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COOPERATING INSTITUTION: University of Nebraska	LOCALITY: Omaha, Nebraska
DIVISION: College of Medicine	DEPARTMENT: Pathology and Bacteriology
OFFICIAL INVESTIGATOR: Dr. Millard F. Gunderson	COLLABORATORS: Dr. Harry W. McFadden, Jr. Georgialee Minthorn
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Bacterial Aspects of Precooked Frozen Foods

SUMMARY

Introduction to literature dealing with the effects of cold on microorganisms.

As a prelude to and in parallel with further experimental investigations concerning "THE EFFECTS OF FREEZING AND STORAGE" on microorganisms in foodstuffs, a review of the literature is undertaken. This review will correlate in abstract and bibliography form, the great maze of material which has gradually accumulated in the literature throughout the years. At the time of this writing, the work is necessarily incomplete; however, further additions to the material here presented will be appended to research reports until the literature on this subject has been adequately reviewed.

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INTRODUCTION.

Although occasional reviews of the literature have appeared concerning some phase of the vast subject "the effects of cold on microorganisms", we have found none which attempt to comprehensively cover the field. During the past decade, the need for storage and transportation of foodstuffs of all types over great distances and in sufficient quantity to serve large population masses has vastly increased. With the greatly increased need, production and research sources have been hard pressed to keep pace; these factors have resulted in a rapidly expanding literature which we feel is now large enough and diverse enough to warrant correlation. It is hoped that this review will prevent needless duplication of effort and will serve as a ready source of material not only for established workers in this field of endeavor but also for new workers entering the field.

As this review is to take an abstract and bibliography form, it is essential that we attempt to classify papers in such manner, that they be presented in logical sequence; consequently, we have divided the papers here reviewed into six broad divisions. Within each division, papers are listed in sequence by publication date so that the older research is presented first and the newer research is presented last.

We have experienced considerable difficulty in placement of some papers within their proper division as there is often vast overlapping of material presented; however, we have done our best to give this review some semblance of order. In some instances, however, papers have been arbitrarily assigned one category when they could as well have been placed in another.

An illustrative example of the methods of reporting our abstracts is as follows:

1. Prudden, T. M.
   1887. On Bacteria in Ice and Their Relations to Disease with Special Reference to the Ice Supply of New York, City. Med. Record, 31;34;-350. (Laboratory of the Alumni Association of the College of Physicians and Surgeons, New York.).

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Body of abstract
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Continued.

DIVISION I is a general section which deals with the effects of cold on microorganisms. In this section are many of the earlier papers and a significant number of papers dealing with the mechanism of destruction of microorganisms by cold per se. We have attempted to place many of the more specialized research papers in this group.

DIVISION II is a specialized section which deals with the resistance of Mycobacterium tuberculosis to cold. This section is included for purposes of completeness; in addition some significant researches are found within the group.

DIVISION III is a large section which deals with the effects of cold on various microorganisms, plants, tissues, and substances other than bacteria. Much basic research is included in this section.

DIVISION IV is a technical section which deals with some of the broader and more general effects of cold on living higher animals, especially man. The purpose of this section is not to review the pathology of frozen limbs or refrigeration anesthesia, but rather to present certain basic phenomena which are of value in a thorough understanding of the effects of cold on life processes.

DIVISION V is an important section which deals with the public health aspects of the food industries, especially as they apply to the frozen food industry. This division is further subdivided into four sections:- (1) General review, (2) Staphylococcic entertoxin studies, (3) intestinal disease studies, and (4) Botulinum studies.

DIVISION VI is an important section which deals with the microbiology of frozen foods without special emphasis on public health aspects, but rather with emphasis placed on some of the problems facing the frozen food industry. This division is further divided into sections according to the type of foodstuff examined; e.g., fruits and vegetables, meats, poultry, etc.

A bibliography, which lists all papers presented, in alphabetical sequence (by author) concludes this report.

There is no claim to originality in this work. As would be expected in many instances, tables, data, and summaries have been incorporated into the review.

DIVISION I

GENERAL EFFECTS OF COLD ON MICROORGANISMS.

1. Prudden, T. M.
1887. On Bacteria in Ice, and Their Relations to Disease, With Special Reference to the Ice Supply of New York City. Med. Record, 31:341-350. (Laboratory of the Alumni Association of the College of Physicians and Surgeons, New York.)

This paper gives in considerable detail numerous experiments undertaken to demonstrate the effects of freezing and thawing on bacteria. Especial emphasis is placed upon its practical applications in considering the ice supply of a great city.

The effect of freezing on separate species of bacteria in distilled water at temperatures between 14 and 30°F was studied. The results are as follows:

	TIME	No. of Bacteria in 1 cc of water.
A.	Bacillus Prodigiosus	
	Before freezing	6,300
	Frozen 4 days	2,970
	Frozen 37 days	22
	Frozen 51 days	0
B.	Proteus vulgaris (Hauser)	
	Before freezing	8,320
	Frozen 18 days	88
	Frozen 51 days	0
	Frozen 65 days	0
C.	Slender fluidifying Bacillus from Croton Water:	
	Before freezing	800,000
	Frozen 7 days	0
	Frozen 18 days	0
	Frozen 38 days	0
	Frozen 47 days	0
D.	Staphylococcus pyogenes aureus	
	Before freezing	Innumerable
	Frozen 18 days	224,598
	Frozen 20 days	46,486
	Frozen 54 days	34,320
	Frozen 66 days	49,280

E. Fluorescent Bacillus from Hudson River Ice

Before freezing	Innumerable
Frozen 4 days	571,560
Frozen 11 days	520,520
Frozen 51 days	183,040
Frozen 65 days	10,978
Frozen 77 days	85,008

F. Bacillus of Typhoid Fever

Before freezing	Innumerable
Frozen 11 days	1,019,403
Frozen 27 days	336,457
Frozen 42 days	89,797
Frozen 69 days	24,276
Frozen 77 days	72,930
Frozen 103 days	7,348

Prudden shows remarkable grasp of his subject as evidenced by the following statement: "The fact that a very considerable reduction in the number of viable individuals occurs at the first freezing, while a more gradual destruction goes on as the low temperature is maintained, is doubtless due to the killing off at once of the more feeble bacteria. Still it would be interesting to know whether the marked rate of destruction is due to this factor alone, or whether the sudden change of temperature may not have a greater effect than a simple prolongation of the unfavorable condition when once the organisms have accustomed themselves to it." Comparative experimental data is given to demonstrate the effect of freezing and thawing.

	Frozen solid and Remaining So.	Frozen Solid, but Repeatedly Thawed and Immediately Refrozen.	
Time	No. of Bacteria in 1 cc of water.	Times Refrozen	No. of Bacteria in 1 cc of water.
A. Typhoid Bacilli (Fresh active culture)			
Before freezing	40,896	- - -	40,896
Frozen 24 hours	29,780	3	90
Frozen 3 days	1,800	5	0
Frozen 4 days	950	6	0
Frozen 5 days	2,490	6	0
B. Staphylococcus pyogenes aureus (Fresh active culture)			
Before freezing	111,782	- - -	111,782
Frozen 15 minutes	52,500	- - -	- - - -
Frozen 2 hours	21,300	- - -	- - - -
Frozen 24 hours	22,690	1	13,495
Frozen 48 hours	6,460	3	110
Frozen 96 hours	6,155	4	0

Continued.

C. Bacillus prodigiosus (Fresh active culture)

Before freezing	339,516	- - -	339,516
Frozen 24 hours	36,41[illegible]	1	2,570
Frozen 30 hours	41,580	2	275
Frozen 48 hours	14,440	3	15
Frozen 96 hours	4,850	4	0

Experiments on the effect of low temperatures on bacteria suspended in water when the water does not crystallize were carried out by the author. "A very considerable destruction of bacteria of various species takes place if the water be placed in a very cold place, but kept just above the freezing point. Thus, in a considerable number of experiments, a much larger number of individuals was destroyed in tubes in which the water did not freeze, although kept several degrees below the freezing point, than in those in which the water became solid." The author attained the supercooled state by coating test tubes with sterile sweet-oil and maintaining them very quietly in a refrigerator at 15 to 28°F. (Note: The greater kill noted by Prudden in the supercooled state than in the frozen state is at variance with the work of later experimenters.)

The implications of the foregoing experimental data are obvious; the author summarizes his data in surprisingly modern fashion. This proved a most interesting and educational review.

2. Prudden, T. M.
1887. On Bacteria in Ice, and Their Relations to Disease, with Special Reference to the Ice Supply of New York City. Med. Record, 31:369-378.

In this portion of his study of bacteria in ice, the author analyses samples of ice coming from rivers, lakes and ponds which supply the City of New York. The author notes the increased number of bacteria found in snow ice and bubbly ice as compared with clear ice and also the increased number of bacteria in contaminated river ice as compared with pond and lake ice. Prudden then goes on to discuss the merits and demerits of the ice supply of New York City.

3. White, A.C.
1899. Liquid Air: Its Application in Medicine and Surgery. Med. Record, 56:109-112.

With the assistance of Dr Parks, the author tested the effects of liquid air (312°F below zero) on typhoid, anthrax, and diphtheria bacilli. The organisms were placed in sealed capillary tubes and then dropped into liquid air. The contents of the tubes were tested after 30, 45, 60, and 90 minutes and in each case good growth was obtained.

4. MacFadyen, A.
1900. On the Influence of the Temperature of Liquid Air on Bacteria. Lancet, 1:849.

Ten organisms (B. typhosus, B. coli communis, B. diphtheriae, Spirillum cholerae Asiaticae, B. proteus vulgaris, B. acidi lacti, B. anthracis, S. pyogenes aureus, B. phosphorescens. and Photobacterium balticum) were exposed to the temperature of liquid air for 20 hours (-182 to -190°C) following which they were thawed and examined. In no instance, whether on solid or liquid medium, could any impairment of the vitality of the microorganisms be detected.

5. Keith, S.C. Jr.
1913. Factors Influencing the Survival of Bacteria at Temperatures in the Vicinity of the Freezing Point of Water. Science, 37:877-879.

This paper gives a general introduction to the problem of the effects of cold on bacteria. Keith's experiments concern a single species of B. coli. Twenty-four hour growth of organisms was suspended in water, normal saline, various dilutions of fat free milk, glycerine, cane sugar, and commercial glucose.

The experimental results may be summarized as follows: (1) When B. coli are frozen in Boston tap water (in test tubes) as solid ice, and held at -20°C, only a fraction of 1% of the original number remain alive at the end of 5 days. Storage of a few weeks results in complete destruction of the bacteria. (2) When B. coli are frozen in Boston tap water not solidly, but as a water ice or sherbet, and held in this condition at -20°C, a large percentage remain alive for many months. (3) When B. coli are frozen in milk, pure and diluted to various degrees with water, the death rate of B. coli increases with the dilution, the largest numbers surviving in the undiluted milk and the fewest in that containing the most water. (4) When suspended in aqueous mixtures containing from 5-40% of chemically pure glycerin and held at -20°C, a very large percentage of B. coli remain alive for at least 6 months. (5) At 37°C, B. coli in water or in 5-20% glycerin die rpidly, few if any remaining alive at the end of 72 hours. The death rate diminishes as the holding temperature is lowered, though it is still marked even just above 0C; but at a temperature slightly lower, a sudden change appears, the death rate at and below that point being but little, if any, greater than at -20°C. (6) By covering a 24 hour growth on agar with sterile 10% cane sugar solution and holding at -10°C, stock cultures of B. subtilis, B. aurococcus, B. megaterium, B. fluorescens, B. proteus, and Sarcina aurantiacus have been kept in a vigorous condition without transfer for 8 months.

From the foregoing data Keith concludes: "Low temperatures alone do not destroy bacteria. On the contrary, they appear to favor bacterial longevity doubtless by diminishing destructive metabolism. Frozen food materials - such as ice cream, milk and egg substance, favor the existence of bacteria at low temperatures, not because they are foods, but apparently because they furnish physical conditions somehow protective of the bacteria." Keith believes this data lends support to the mechanical destruction theory of bacteria at low temperatures for in substances - such as pure water - which crystallize solidly, bacterial death is increased as compared with the death of bacteria in substances which crystallize more loosely.

6. Hilliard, C. M., Torossian, C., and Stone, R.
1915. Notes on the Factors Involved in the Germicidal Effect of Freezing and Low Temperatures. Science, 42:770-771. (Simmons College)

This paper gives a brief review of some of the earlier work concerning the effect of cold on microorganisms and then proceeds to a discussion of some of the variables which must be considered in experimentation on such a subject.

Preliminary experimental results are reported. (1.) When B. coli and B. subtilis are frozen in tap water for 3 hours, some 99% of B. coli are killed and some 80% of B. subtilis are killed. (2.) Intermittant freezing has but slightly greater germicidal value than has sustained freezing for the same period of 3 hours. (3.) Tubes containing the bacteria were frozen and held for 3 hours for comparison at approximately -15°C and 2°C. The cold temperature was considerably more fatal. (4.) Using cream containing 30% butterfat, the authors found very striking protection afforded the bacteria when frozen, whether the freezing was continuous or intermittent. Freezing and thawing was considerably more fatal than continuous freezing.

7. Albert, H., Hinman, J. J., and Jordan, D. G.
1916. Bacterial Changes in Uniced Specimens of Water. J. Bact., 1:113.

This paper deals with the reliance which may be placed on bacteriological analyses of water after 8, 24, 48, and 72 hours in the uniced state.

8. Sedgwick, W. T., Hamilton, H. W., and Funk, F. J.
1917. Experimental studies on the Effect of Various Solutions upon the Viability of Bacteria at Low Temperatures. Abst. Bact. 1:49.

In considering the viability of bacteria at low temperatures, the important factors to be studied are as follows: (1) the nature of the solution or medium, (2) the species of the organism, and (3) the temperature. This holds true at higher temperatures but the

effect is often quite reversed in solidified tubes, the organisms surviving longer in solutions which are quite toxic at the usual temperatures. The mechanical protection given bacteria from sheering ice crystals by solutes is important.

9. Hilliard, C. M., and Davis, M. A.
1918. The Germicidal Action of Freezing Temperatures Upon Bacteria. J. Bact., 3:423-431. (Simmons College, Boston Mass.)

This paper contains an excellent review of the earlier literature. Temperature is one of the cardinal factors influencing micro-organisms. The majority of bacteria are unable to exercise normal metabolism at temperatures below 6°C or above 45°C. The destructive influence of high temperatures and other physical and chemical agents proceeds in an orderly and predictable manner. Cold as a disinfectant seems to be an exception to this rule; as metabolism is arrested to its lowest ebb by temperatures lower than the minimum, survival is theoretically, at its maximum. If the temperature is depressed to the freezing state, new factors come into play and it is with these that this paper deals.

The authors drew the following conclusions (noting, however, that their work is not extensive enough to render a final statement.) (1) Intermittant freezing of bacteria exerts a more effective germicidal action than continuous freezing. (2) The reduction is much less in milk and cream than in pure tap water when freezing temperatures are applied, due, no doubt, to physical protection offered to the bacteria by the colloidal and solid matter in suspension. (3) The degree of cold below freezing is not a very important factor in the destruction of bacteria. There is no critical temperature below freezing where the germicidal effect is greatly accelerated. (4) The death rate of B. coli is much higher in media which are frozen solid than it is in the same medium not solid and at a slightly lower temperature. (5) Crystallization, probably resulting in mechanical crushing, is an important germicidal factor in causing the death of bacteria at zero degrees Centigrade and below. The greatest reduction occurs promptly upon freezing and refreezing, but is not caused so much by the sudden change in temperature as by this mechanical factor.

10. Prucha, M. J. and Brannon, J. M.
1926. Viability of Bacterium Typhosum in ice cream. J. Bact., 11: 27-29. (Division of Dairy Bacteriology, Department of Dairy Husbandry, University of Illinois.).

One gallon of ice cream mix - - containing 12% sugar, 12% solids other than fat, and 10% fat - - was prepared, sterilized, and inoculated with Bacterium typhosum, and then incubated until the bacterial count was about 25,000,000 per cc. The mixture was then frozen and stored in a room at a temperature ranging between 8° above to 8° below zero F. Samples were analyzed from time to time.

Samples taken	Typhoid bacteria per cubic centimeter of Ice Cream
Before freezing	25,000,000
Freshly frozen	51,000,000
5 days old	10,000,000
12 days old	7,000,000
20 days old	2,200,000
70 days old	660,000
104 days old	900,000
134 days old	210,000
165 days old	640,000
170 dyas old	711,000
200 days old	60,000
260 days old	67,000
290 days old	53,000
342 days old	51,000
430 days old	30,000
544 days old	13,000
648 days old	11,000
2 years	6,300
2 years, 4 months	living typhoid organisms present.

11. Ford, W. W.
1927. Textbook of Bacteriology, page 154.

This text merely states: "cold of itself has little bactericidal action. Bacteria resist freezing and thawing and are usually not destroyed by the temperature of liquid air, -190°C".

12. Rivers, T. M.
1927. Effect of Repeated Freezing (-185°C) and Thawing on Colon Bacilli, Virus III, Vaccine Virus, Herpes Virus, Bacteriophage, Complement, and Trypsin. J. Exper. Med. 45:11-21. (Hospital of the Rockefeller Institute for Medical Research).

The author reviews some of the pertinent literature, particularly as regards virus and tissue studies at extremely low temperatures. The experimental temperature of -185°C. was attained by the use of liquid air. Tables which summarize the experimental results obtained follow:

TABLE I

Summary of Experiment Showing the Effect of Dilution and Diluent on the Percentage of Colon Bacilli Killed by Freezing and Thawing Four successive times.

Diluent	Dilution	No. bacteria/cc before freezing	No. bacteria/cc after 4 freezings and thawings.
Locke's solution	1-10	18,400,000	27,000
Locke's solution	1-1000	380,000	20
Broth	1-10	44,000,000	13,200,000
Broth	1-1000	600,000	188,000

In another experiment colon bacilli, washed from an agar slant and suspended in broth, were frozen and thawed 12 consecutive times. There were 180,000,000 viable organisms/cc. initially; after freezing and thawing only 40,000 organisms/cc. remained viable.

TABLE II

Summary of Results Obtained by Repeated Freezing and Thawing of Virus III, *

No. of freezings and thawings	1-10	1-100	1-1000
Control (unfrozen)	++	+±	+
2	++	+	±
12	-	-	-
22	-	-	-

* pluses indicate the presence of a virus reaction at the sites of inoculation; minuses indicate the absence of a reaction. Dilutions are in terms of a stock testicular emulsion containing the virus.

TABLE III

Summary of the Results Obtained by Repeated Freezing and Thawing of Vaccine virus. *

No. of freezings and thawings	1-1000	1-10000	1-100000
Control (unfrozen)	+ + +	+ +	+
24	+ +	+	+
34	+ +	+	-

* See Table II.

The author states: "The activity of herpes virus in a fresh stock emulsion was not appreciably decreased by 12 successive freezings and thawings." The author enlarges this statement, however, and goes on to state that the virus is susceptible to such treatment under certain conditions.

The titer of complement in undiluted serum and in serum diluted 1-10 is apparently not appreciably decreased by 12 successive freezings and thawings; however, complement in highly diluted serum is inactivated by repeated freezing and thawing.

Following experiments with trypsin, the author concludes that trypsin is inactivated by repeated freezing and thawing, and under the conditions studied, a greater percentage is inactivated in a dilute rather than in a concentrated solution.

13. Tanner, F. W. and Wallace, G. I.
1931. Effect of Freezing on Microorganisms in Various Menstra. Proc. Soc. Exper. Biol. & Med., 29:32-34.
(Dept. of Bact., University of Illinois, Urbana).

After studying the behavior of microorganisms in a large number of commercially packed frozen fruits and vegetables, the authors conclude that although the numbers of viable organisms showed considerable reduction, there was no sterility even after storage at -16°C. for two years. Eighteen pure cultures maintained in various media for a period of 19 months showed the same results (of the strains studied, E. coli and the molds showed greatest reduction; B. subtilis showed greatest resistance).

Increasing the acidity of the menstrum seemed to increase destruction.

A high concentration of sodium chloride (6%) seemed to increase destruction.

Non-spore forming bacteria, especially when frozen in acid medium, were completely destroyed in from 5-10 months.

Alternate freezing and thawing was more destructive than continuous freezing.

Pure cultures of microorganisms were held in distilled water and cherry juice at temperatures of -16°C., -40°C., and -79°C. In distilled water there was no noticeable difference in the death rates at the different temperatures. Escherichia coli in cherry juice showed slightly greater longevity at the coldest temperature.

Experiments with Clostridium botulinum in several vegetables and fruits showed that spores of the organism survived freezing at -16°C. for 14 months. The toxin showed no decrease in toxicity when stored at -79°C. for 2 months or at -16°C. for 14 months. Vegetables to which detoxified spores were added prior to freezing at -14°C. for 14 months, became toxic in from 3-6 days when allowed to thaw and stand at room temperature. With frozen fruits, despite a pH which ordinarily prevents toxin production, toxin was formed in a few instances -- possibly due to the concomitant development of molds.

Colon-typhoid type organisms died out in cherry juice when held at -14°C. for 2 weeks; however, when held at -16°C in the presence of both cherries and juice, the organisms were identifiable at the end of 5 months.

14. Hampil, Bettylee
1932. The Influence of Temperature on the Life Processes and Death of Bacteria. Quart. Rev. Biol., 7:172-196. (Dept. of Bact., School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.)

This paper presents a rather exhaustive review of the effects of temperature on the life and death of bacteria. A long section of this article deals with normal growth and death of bacteria and the effects of high temperature on bacteria. Some of the generalizations on the effects of low temperature on bacteria are worthy of consideration -- (1) Spores, because of their low water content, remain almost unaffected by low or freezing temperatures. Vegetative forms of different species, however, are known to vary in their reactions to an unfavorable environment. Some bacterial cultures die off rapidly when placed at temperatures 5-10 degrees below their optimum for growth; other organisms are known to multiply regularly though slowly. (2) The preservation of bacterial cultures by storing at low temperatures has been practiced since the beginning of bacteriology, and it has been assumed that such preservation is due to cessation of metabolism on the part of the organisms. (3) It is also recognized that freezing does not destroy bacteria, especially when the change is brought about rapidly. On the other hand alternate freezing and thawing disrupts the relation of the water and the protein molecules to such an extent that death is more apt to occur than when freezing temperatures are maintained over a long period of time.

Continued.

The author presents a compendium entitled "The Viability of Certain Mesophiles at Low Temperature" in which she lists the findings of various workers concerning 13 species of bacteria. This compendium is a quick source for considerable data of general and historical interest.

15. Rake, G.
1935. Viability and Virulence of Frozen and Dried Culture of Meningococcus. Proc. Soc. Exper. Biol. & Med., 32; 975-977. (Laboratories of the Rockefellar Institute for Medical Research, New York, New York.).

This author presents a method of freezing and drying of meningococci by which virulence and viability are retained.

16. Tanner, F. and Schneider, D.
1935. Effect of Temperature on the Storage of Bacteria in Water Samples. Proc. Soc. Exper. Biol. & Med., 32; 960-965. (Dept. of Bact., University of Illinois, Urbana).

Data collected in this investigation indicates in practically all cases that water samples should be kept cold between collection and analysis. This would indicate the icing of samples as provided in Standard Methods for the Examination of Water and Sewage is desirable.

17. McCulloch, E. C.
1936. Disinfection and Sterilization, Lea and Febiger, Philadelphia, Pages 119-126.

This author states that "with few exceptions cold allows the passage of time without the changes which would ordinarily take place and bacteria live longer. Cold protects bacteria from natural as well as chemical agents." In the body of the paper the author develops his stated thesis by enumerating experimental works on pathogenic bacteria, filterable viruses, and the like which are able to withstand prolonged periods of freezing and storage. He mentions the wll known fact that certain organisms - - as Meningococci, Gonococci, and possibly Lactobacillus acidophilus - - are extremely sensitive to cold.

Some of the technical problems of refrigeration are reviewed in this paper.

18. Dubos, R. J.
1937. Mechanism of the Lysesof Pneumococci by Freezing and Thawing, Bile, and Other Agents. J. Exper. Med. 66;101-112. (Hospital of the Rockefellar Institute for Medical Research).

Bile has the property of killing rapidly, and lysing completely, the cells of most strains of pneumococci. This breaking up of pneumococci can also be achieved by repeated freezing and thawing

of the cells. The stress due to the formation of intracellular ice crystals has been regarded as causing the disruption of the cells; however, it is a matter of great importance whether the techniques used for breaking up pneumococci involve only a change in colloidal state (peptizing action of bile salts and fatty acids, mechanical disruption by ice crystals), or whether they bring about more profound changes of the components of the cellular structure.

Various experiments presented by the author show that to be soluble in bile, or liable to disruption by freezing and thawing, the pneumococci, living or dead, must still possess their autolytic enzymes in a potentially active form. An interesting and significant discussion develops this thesis and points out many problems as yet to be solved.

19. Haines, R. B.
1937-38. The Effect of Freezing on Bacteria. Proc. Roy. Soc. (Lond.)., B. 124:451-463. (Low Temperatue Research Station, Cambridge.).

This paper, in our opinion, is especially pertinent with regard to the mechanisms occurring during freezing and thawing; hence, the comments of the author are reproduced in some detail. The author experimented with Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Achromobacter, Saccharomyces cerevissiae, Bacillus mesentericus, Bacillus cereus, and Bacillus megatherium frozen at variable temperatures in tube and cover slip preparations. His comment is reproduced at some length as follows:

"Two aspects of the effect of freezing on bacteria can be observed. First, when bacterial suspensions are frozen rapidly at -70°C, and thawed a certain proportion of the cells is killed, varying from about 80% with the most susceptible organism, B. pyocyaneus, to little or none with spores. The mortality is constant under these conditions with young cells, but varies somewhat with the manner of freezing. The rate or temperature of freezing appears to have little effect. Secondly, when the quickly frozen suspensions are stored at such temperatures as -1, -2, -5, -10 and -20°C., the rate of death again varies with the organism, being most rapid with B.pyocyaneus and little or none with spores. With some organisms, notably B. pyocyaneus and B. coli rapid death occurs near the highest temperature of storage and comparatively slow death at -20°C. When there is any difference in the rate of death between the upper and lower temperatures, it is always in this order.

In attempting to explain these observations, frozen and unfrozen cells were examined by dark ground illumination and in stained preparation. It is often stated that mechanical destruction of bacteria when frozen in bulk can be obtained with repeated freezing and thawing (e.g. Young, 1929) but no evidence for its occurrence, either in the single freezing or after storage in the frozen state, could be found under the conditions of the present experiments. Damage to the cellular membrane, leading to sufficiently increased permeability for the liberation of enzymes, might, however, well escape observation. Turning to the effects of freezing on the proteins of the bacterial cell, it was found that rapid flocculation of one protein fraction of an extract of B. pyocyaneus, prepared at low temperatures to obtain the "native" proteins, occurred at -2°C. Such flocculation took place slowly, if at all, at -20°C. It seems likely, therefore, that the death of bacteria in the frozen state is due to some change, or complex of changes, in one moiety of the cellular proteins leading to denaturation and subsequent flocculation, owing to the particular salt concentration and pH obtained. This observation probably furnishes the explanation of the success of methods of rapid freezing, with desiccation, for the storage of bacteria and sera, since the cellular protein most sensitive to changes in environment can be obtained in an undenatured condition by extraction at low temperatures, and keeps indefinitely when dry.

No flocculation could be demonstrated on a single freezing and thawing, and yet with some organisms a big mortality occurs during this process. A similar phenomenon has, however, been found in muscle (op.cit.) and the explanation put forward by Hardy (1928) of the occurrence of a time-lag, may hold here. The picture suggested is that some change is brought about in the organization of the cellular proteins, either denaturation itself or some change leading to denaturation, by the unfavorable environment set up at temperatures just below zero. The proteins, or one fraction, are then subsequently flocculated, and this flocculation can be measured. If the critical zone of temperature is passed through rapidly, less damage is done to the cell. If this view is correct, it suggests that the resistance of spores is due to the fact that their cells do not contain the particular protein most sensitive to changes in environment. This point is under investigation in a more extended study of the proteins of bacteria."

20. McFarlane, V. H.
1938. Behavior of Microorganisms at Subfreezing Temperatures. A Thesis Submitted for the Degree of Doctor of Philosophy, University of Washington).

This thesis seems especially well done and should probably be thoroughly studied by research workers in this field of endeavor; however, we will try to summarize and point out some of the highlights of the paper. The methods and goal of the study are found in the introductory remarks and may be summarized as follows: (1) Food spoilage may be brought about by enzymatic, chemical, and or microbial changes. Physical changes may also occur in frozen foods which influence their palatability. (2) The danger of persistence of microbial life (food spoilage or pathogen) is obvious. (3) "The conception held in the present work is that within the frozen mass (water, syrup, brine, juice, syrup-packed fruit, brine-packed vegetable) conditions are far from homogeneous. Such factors as manner and rate of crystallization, density and viscosity of the medium, gravity, mechanical obstructions, etc., all tend to influence redistribution of suspended and dissolved substances in a fluid medium during the freezing process. The assumption seems warranted that if the conditions are not uniform throughout the frozen mass, there may be within the mass certain areas especially favorable for microbial survival or microbial destruction. These conditions would arise most freely when fluid media are frozen solidly in undisturbed containers, and would prevail under such conditions as are common in frozen pack practice." (4) The pure cultures used were a Saccharomyces species isolated from cider and Escherichia coli. Some natural organisms were used in studies of cider, raspberries, and peas. (5) Temperatures of -20°C, and -10°C. were used in the studies.

Results obtained in the several experiments conducted indicate: (1) Soluble and suspended substances tend to be concentrated in the elongated core areas of the frozen mass and in the upper horizontal layer so that unbuffered solution originally pH3.22, showed in the frozen mass a range of pH from 2.49 in the core to 6.11 in the periphery. In a 10% sucrose solution, pH 3.22, the freezing concentration of hydrogen ions showed a pH range from 2.57 to 4.09. In apple cider and frozen pack fruits and vegetables the freezing redistribution of hydrogen ions is less marked rarely varying more than 0.2 in pH. (3) In studies with yeast cells, there was a tendency for viability to decrease progressively with passage of time; the rate of decrease varied with the nature of the suspending medium and the temperature. Greater retention of yeast cell viability occurred at -20°C. than at -10°C. when other conditions were identical. (4) Decrease of viable microbial forms in cider frozen and stored at -20°C, and -10°C was progressive.

Continued.

Greatest preservation occurred in cider at the higher temperature. (5) Decrease of viable microorganisms was progressive in brine-packed peas and syrup-packed raspberries when stored at -20°C and -10°C. Greatest destruction occurred at the higher temperature. (6) Increasing the concentration of sucrose in the storage medium in general protected the organisms studied from effects of freezing and storage.

21. McFarlane, V. H.
1941. Behavior of Microorganisms at Subfreezing Temperatures. III. Influence of Sucrose and Hydrogen-Ion Concentrations. Food Research, 6:481-492. (Agriculture Chemical Research Division, Bureau of Agriculture Chemistry and Engineering, U. S. Dept. of Agriculture, Washington,D.C.)

Sucrose syrup is used in the frozen food industry in the preparation of fruits for dessert purposes. Aside from the sweetening property this syrup has decided value in preserving the color, texture, appearance, and general palatability of the product. Unfortunately large numbers of molds, yeasts and bacteria, especially spore-forming species of this latter group, are capable of surviving long periods of exposure to low temperature in frozen, syrup-packed fruits. Experimental data is presented on the viability of a Saccharomyces species and Escherichia coli suspended in water and in sucrose solutions at -10°C and -20°C.

Higher concentrations of sucrose, 30 and 50 percents, tended to retard destruction of the two microorganisms. When the hydrogen-ion concentration was the only variable, a reaction of pH 6.5 was found to be more favorable than a reaction of pH 5.0 for hastening the destruction of yeast cells in some of the sucrose media; but in the case of E. coli the experimental evidence indicated greater destruction occurred in those samples which possessed the greater hydrogen-ion concentration. Of the conditions investigated the most destructive for both microorganisms was pH 3.6 to 3.7. When temperature was the only variable, greater kills, after several weeks' storage, tended to occur at -10°C. than at -20°C.

22. Stillman, E. G.
1941. Preservation of Pneumococcus by Freezing and Drying. J. Bact., 42:689-693. (Hospital of the Rockefeller Institute for Medical Research, New York.).

Pneumococci in the rapidly frozen and dried state may remain viable for at least 3 years. The serological specificity and virulence of strains recovered after freezing and drying remain unaltered. Variations in the viability of the different types of pneumococci were observed under the conditions studied.

23. Kessler, W. R.
1942. Preservation of Bartonella muris in Frozen State. Proc. Soc. Exp. Biol. & Med., 49;238-241.

The infectivity of Bartonella muris can be preserved for at least 11 weeks when infected defibrinated rat blood is rapidly frozen in a mixture of dry ice and alcohol and then preserved in dry ice.

24. Deakin, R.
1942. Preservation (by Quick Freezing) of Gonococcus in Urines and Broths. Am. J. Syph., Gonor., and Ven. Dis., 26;313-315. (Dept. of Bact., Washington University and the Washington University Clinics, St. Louis, Missouri.)

Gonococci were preserved for from 24 to 55 hours by freezing in urine and infusion broth in absolute alcohol and dry ice. The purpose of the study was to prove whether cultures of this organism could be shipped to central laboratories. Thawing of the specimen must be accomplished just before plating.

25. Castell, C. H. and McDermott, L. A.
1942. Multiplication of Bacteria in Water and Its Significance in Food Spoilage. Food Research, 7;244-253. (Dept. of Bacteriology, Ontario Agricultural College, Guelph, Canada.).

"Water is essential in almost all food industries and if the bacterial flora of water can increase from dozens to millions simply by remaining at ordinary temperatures for a day or so, this may have great significance quite apart from the public health stanpoint."

In their experiments the authors used driven well water which was unchlorinated and contained an extremely low bacterial count per milliliter. Water was held at room temperature (25°C.) and plated on beef extract agar with the following results - - initial 4/cc; 48 hours 1,972,000/cc; 6 days 223,000/cc; 40 days 300,000/cc; and 60 days 40,900/cc.

Samples of water were then held at three representative temperatures - - 25°C., 37°C., and 2-5°C. In each case bacterial multiplication occurred as proved by plate count techniques. In the case of the samples held at 2-5°C. the results were unpredictable and quite variable; however, multiplication did occur. During the course of this study the authors made the observation that the microflora which develops in water held at room temperature has a temperature range reaching almost to the freezing point.

In the course of their studies the authors noted that many organisms will grow in distilled water; that chlorine and sodium chloride inhibit the growth of bacteria; and that the faster the flow of water the lower the count.

"The following species showed active multiplication in water, reaching counts of over 500,000 per ml.: Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas fragi, Aerobacter aerogenes, Serratia marcescens, and Achromobacter lipolyticum. Pseudomonas putrefaciens gave variable results but apparently grew better in water when accompanied by certain other organisms." "Escherichia coli, Proteus vulgaris, Alcaligenes viscosus, Staphylococcus aureus, Staphylococcus citreus, Sarcina lutea, Micrococcus conglomeratus, Bacillus subtilis, Bacillus mycoides, Bacillus graveolens, Bacillus panis, and Bacillus mesentericus showed no significant increase over a period of 20 days."

26. Nelson, F. E.
1944. Factors which Influence Growth of Heat-Treated Bacteria. J. Bact., 48:473-477. (Kansas Agricultural Experiment Station, Manhattan, Kansas.).

The author concludes that his investigations furnish further evidence that heat treated bacteria are more exacting in their requirements for initiation of growth than are unheated control bacteria. Not only the presence of adequate kinds of nutrients but also the quantities of these nutrients and the order in which the peptone supplement is added to the basal medium seem to be of significance in determining the viable population in a heat treated culture.

(Note: Does a similar state occur in cold-treated bacteria? This might lead to a study of suitable media to be used in determining viable populations in frozen products.).

27. Weiser, R. S. and Osterud, C. M.
1945. Studies on Death of Bacteria at Low Temperatures; Influence of Intensity of Freezing Temperatures, Repeated Fluctuations of Temperature, and Period of Exposure to Freezing Temperatures on Mortality of Escherichia coli. J. Bact., 50:413-439. (University of Washington, Seattle, Washington.).

This investigation was undertaken to assemble reliable quantitative data on the death of bacteria at low temperatures which would be of value in elucidating the manner in which low temperature injury is produced. The investigation was limited to the study of Escherichia coli suspended in 1% peptone or a peptone buffer mixture at pH7.0.

The following valuable conclusions are included in the summary: (1) Death by freezing involves rapidly acting or "immediate" death, caused by freezing and thawing per se, and a "storage" death which is a direct function of the time and temperature. (2) Immediate mortality by freezing is marked but does not vary with the intensity of the freezing temperature. (3) Immediate death occurs at a brief stage in the freezing process during which extracellular ice formation is being completed. (4) The rate of storage death at the higher freezing temperatures is very rapid and is much greater at temperatures above -30°C than at temperatures of -30°C and below. (5) Repeated freezing is more lethal than a single freezing and thawing or storage in the frozen state for a similar interval of time. (6) Freezing is more lethal than supercooling. (7) Repeated fluctuations of temperature of frozen suspensions do not exert a lethal action additional to that of storage. (8) Repeated fluctuations of temperature of frozen suspensions between -30°C and -78°C appear to result in a lower mortality than storage at either temperature; however, this protective effect was not noted at temperatures above -30°C nor below -78°C. (9) Storage death at 195°C either does not take place or is so slow that it is difficult to detect within the storage period studied.

In their discussion the authors indicate that the weight of their experimental evidence here presented supports the theory of the mechanical action of extracellular ice as the principal cause of immediate death due to freezing, as opposed to the theory of intracellular ice formation, or the theory of concentration of solutes as the ice crystals are formed. This discussion is interesting and seemingly pertinent.

28. Weiser, R. S. and Hargiss, C. O.
1946. Studies on the Death of Bacteria at Low Temperatures; the Comparative Effects of Crystallization, Vitromelting, and Devitrification on the Mortality of Escherichia coli. J. Bact., 52; 71-79. (Dept. of Bacteriology, University of Washington, School of Medicine, Seattle, Washington.).

This paper is a continuation of the 1945 experiment, and is an attempt to gain additional information concerning the mechanism of death of bacteria by freezing.

"Water, if cooled to very low temperatures under special conditions, may crystallize without forming ice. Briefly vitrification of any aqueous solution can be accomplished by reducing the temperature through the zone at which crystallization occurs so rapidly that there is insufficient time for crystals to form. Increasing the viscosity usually aids the ease of fibrification. If vitreous water is warmed slowly, devitrification will occur; whereas, if it is warmed rapidly vitromelting will occur. Slow warming does not result in a change to the crystalline state (vitrification) until temperatures approaching 0°C. are reached.

In their summary the authors conclude: (1) "Escherichia coli was suspended in 10% sucrose and subjected to crystallization, vitromelting, and devitrification treatments at -195°C. The vitromelting was found to be more lethal than crystallization treatment. (2) The devitrification treatment was more lethal than either the vitromelting or the crystallization treatment."

In their discussion the work of Stiles (1930); Luyet and Gehenio (1940); Goetz, A. and Goetz, S. and Breedis (1942) are mentioned. The authors then present their theories concerning the mechanism of death of bacteria by low temperatures.

29. Naylor, H. B. and Smith, P. A.
1946. Factors Affecting the Viability of Serratia marcescens During Dehydration and Storage. J. Bact., 52;565-573. (Camp Detrick, Frederick, Maryland.).

To obtain a maximum survival of Serratia marcescens during dehydration and storage, the authors report the following conditions are most satisfactory: (1) Harvest the cells at the end of the logarithmic growth phase from an aerated culture (18-24 hours at 30-34°C). (2) Mix the cell concentrate with a solution containing ascorbic acid, thiourea, NH_4CL, and dextrin at pH6-7. (Optimum concentration 0.5, 0.5, 0.5, and 2.0 respectively.) (3) Dehydrate by lyophilization. (4) Store in a high vacuum.

DIVISION II.

RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS TO COLD.

1. Cornet, G.
   1904. Tuberculosis and Acute Generalized Tuberculosis. American Edition by W. B. James and A. Stengel Pages 45-46.

Resistance to Cold - - The tubercle bacilli have the power of resisting cold for a long period. In Galtier's experiments even temporary freezing did not destroy their virulence. During the winter of 1888, the author (Cornet) allowed some tubercuous sputum to dry upon asphalt plates in the narrow yard of the institute. "In a few days it snowed, and the frost often fell as low as -10°C; the snow lay for about 3 weeks. In the 5th week there was a second snowfall, lasting several days. Every week, from the first to the sixth, sputum specimens were taken and inoculated upon guinea-pigs. Every time, even after the sixth week, the sputum was found to be of full virulence. As these experiments were undertaken for a different purpose, accurate temperature records were not kept, yet they suffice to show that tubercle bacilli retain their vitality as long as six weeks at very low temperatures, up to -10°C, under a covering of snow."

2. Twitchell, D.
   1905. The Viability of Tubercle Bacilli in Sputum. Med. News, 87:642-647. (M.I. Saranac Lake, New York.)

The purpose of this experimentation was to prove how long tubercle bacilli would live in sputum under natural conditions. The sputum used in the studies was a mixture of that obtained from two patients with advancing pulmonary tuberculosis. The work may be divided into seven portions - - the first three portions deal with the exposure of test sputum to moisture, diffuse light, direct sunlight, darkness, and moderate heat; the fourth portion deals with the exposure of test sputum to ordinary living conditions when exposed on a handkerchief, a carpet, on wood, and on a woolen blanket; and the last three portions deal with the exposure of test sputum to conditions of cold and freezing.

The results of the first four portions of the experiment are given in considerable detail; however, they may be summarized as follows: tubercle bacilli will live for moderately long periods of time (as long as 170 days) in the materials tested. This organism is particularly favored by moisture and darkness and is destroyed in relatively short time by direct sunlight. The results of the work with cold are given in more detail as follows: (1) sputum within open white glass bottles, stored outdoors, during the winter months produced typical tuberculosis in test guinea pigs after 110 days but not after

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131 days, (2) sputum within blocks of ice produced typical tuberculosis when thawed and inoculated into test guinea pigs after 102 days but not after 136 days.

3. Gloyne, S. R.
1920. A Note on the Viability of Acid-Fast Bacilli. Tubercle, 2:12-13. (Pathologist, City of London Hospital for Diseases of the Chest.).

The author noted that one stock culture of tubercle bacillus on Dorset's egg medium survived untouched for nearly six years, while two cultures on glycerin agar died out.

4. Corper, H. J. and Gauss, H.
1923. The Preservation of Cultures of Human and Bovine Tubercle Bacilli. Am. Rev. Tubero., 6:1040-1045.

Fully grown cultures of human tubercle bacilli may remain viable on Petroff's gentian violet medium or 5% glycerol-agar, whether kept in the ice box or incubator, for from 4-8 months. Bovine tubercle bacilli are more resistant and may remain viable from 8-16 months. Drying tends to shorten the period of viability.

5. Shope, R. F.
1926. The Survival of the Tubercle Bacillus in Suspension in Physiological Salt Solution. J. Exper. Med. 44:623-624. (Dept. of Animal Path. of the Rockefeller Institute for Medical Research, Princeton, N.J.).

This note was to place on record the observation that three suspensions of tubercle bacilli in physiological saline were alive and virulent for guinea pigs after standing for periods of 310, 325, and 330 days respectively, at refrigerator temperature. The temperature of the refrigerator fluctuated about the freezing point but was not recorded. All of the test guinea pigs showed a well marked generalized tuberculosis and there was nothing atypical in the course of the disease or in the autopsy findings.

6. Gloyne, S. R.
1928. The Viability of the Tubercle Bacillus Under Zero Conditions Outside the Body. Tubercle, 9:573.

A small portion of growth of a human strain of tubercle bacillus of known virulence was removed from a culture on Dorset's egg medium. This portion of growth was transferred to a Hayden's mortar, ground up with triple distilled water, diluted to a strength of 1,000 million bacilli per cubic centimeter and placed in vaccine ampoules which had been previously washed with triple distilled water and sterilized.

The ampoules were then sealed and packed with ice in the interior of a vacuum flask. The flask was replenished with ice daily. Except for a few moments during the refilling of the flask with ice, the ampoules remained constantly in the dark. Each week for twelve weeks an ampoule was removed and the contents inoculated subcutaneously into a test guinea pig. The contents of all twelve ampoules produced generalized tuberculosis in guinea pigs. Negative results were apparently not obtained and the experiment was terminated at the end of twelve weeks.

7. Williams, R. S. and Hoy, W. A.
1930. The Viability of B. tuberculosis (bovinus) on Pasture Land, in Stored Faeces and in Liquid Manure. J. Hyg., 30;413-419. (National Institute for Research in Dairying, University of Reading)

Under ordinary conditions B. tuberculosis (on pasture land in the south of England) may remain alive and virulent in cow's feces for a period of five months in winter, two months in the spring , and for four months during the autumn. During the summer no living organisms were demonstrated after two months. Studies of viability in stored feces and liquid manure showed the organism to be very resistant.

8. Harris, M. M. and Lange, L. B.
1932-33. A Note on the Preservation of Acid-Fast Bacteria in Vacuo. J. Lab. & Clin. Med., 18;1066-1067. (Dept. of Bact., The John Hopkins School of Hygiene and Public Health).

A "Brown's" modified method for preservation of acid fast bacteria is described. Organisms were preserved for as long as 11 months by this method. (A relatively simple method).

9. Corper, H. J. and Cohn, M. L.
1933. The Viability and Virulence of Old Cultures of Tubercle Bacilli, Studies on Twelve-Year Broth Cultures Maintained at Incubator Temperature. Am. Rev. Tuberc., 28;856-874.

A readable report on prolonged viability and virulence of tubercle organisms.

10. Cohn, M. L.
1939. Preservation of Tubercle Bacilli. Am. Rev. Tuberc., 40;99-108. (Research Dept., National Jewish Hospital, Denver.).

Desiccated human (virulent and avirulent), bovine (virulent), and avian (virulent) tubercle bacilli almost completely retain viability for three years at refrigerator temperature (3°C),

while natural cultures survive under the same condition of temperature only about six months to one year (occasionally two years). The loss of viability of the desiccated cultures of these same strains of tubercle bacilli is much more rapid at incubator temperature, being almost complete at six months to one year; while at room temperature, they survive a little better than at incubator temperature. The loss of viability of tubercle bacilli is primarily a function of the temperature at which they are stored, regardless whether they are desiccated or natural.

11. Kyes, P. and Potter, T.
1939. The Resistance of Avian Tubercle Bacilli to Low Temperatures with Especial Reference to Multiple Changes in Temperature. J. Infect. Dis., 64:123-134. (Lab. of Preventive Medicine, University of Chicago.).

In this paper the authors give a review of the earlier literature as regards the destructive effect of low temperatures upon living matter in general, on bacteria, and most particularly on tubercle bacilli. Kyes and Potter (this paper) demonstrate that avian tubercle bacilli may not only survive the temperature of liquid air but even several freezings as practiced by Swithinbank, and that they may survive when rapid freezing and thawing is accomplished 20, 40, 80, and even 200 times. One experiment shows a similar result when freezing and thawing are accomplished at a distinctly slow rate. Although bacilli are invariably present after freezing and thawing, their ability to multiply in culture and to produce rapidly progressive disease in susceptible animals is distinctly reduced.

12. Glover, R. E.
1946. Effect of Freeze-Drying and Low Temperatures on Viability of Mycobacterium tuberculosis. J. Path. & Bact., 58:111-114.

Human and bovine strains of M. tuberculosis suspended in distilled water, normal saline, and inactivated bovine serum were preserved at a temperature of -76°C and by freeze-drying. The number of viable bacilli in tenfold dilutions was ascertained before treatment and again at varying intervals up to 180 days by the inoculation of guinea pigs and hamsters and by cultural methods. Cultures stored at low temperature showed no appreciable loss after 180 days; freeze dried material, however, sustained an immediate fall in activity, estimated at 100 to 1000 fold; thereafter, the dried material remained stable.

DIVISION III.

EFFECT OF COLD ON ORGANISMS OTHER THAN BACTERIA.

1. Boak, R. A., Carpenter, C. M., and Warren, S. L.
1933. The In Vivi Thermal Death Time of Treponema Pallidum. J. Bact., 25:93. (Proceedings Society of American Bacteriologist).

The in vitro thermal death time for Treponema pallidum has been measured at:-

5 hours at 39°C
3 hours at 40°C
2 hours at 41°C
1 hour at 41.5°C.

The length of time required to kill Treponema pallidum in the body of experimental rabbits by fever temperatures checked fairly well with the thermal death time obtained in vitro; however, it was slightly longer at each temperature.

2. Turner, T. B. and Brayton, N.L.
1939. Factors Influencing the Survival of Spirochetes in the Frozen State. J. Exper. Med., 70:639-650. (Dept. of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.)

Titration experiments with relapsing fever spirochetes before and after freezing showed the following results: (1) With each freezing and thawing there was a slight but regular decrease in virulence, which decrease bears no relation to the duration of storage at -78°C. Ordinarily infectivity is destroyed by more than four freezings. (2) Motility was not always a good criterian of infectivity. (3) Cooling spirochetes from 0°C to -78°C over a 2-6 hour period damages them only slighly more than does rapid cooling, but warming over a 2-6 hour period kills most of the organismsm Rapid thawing, as in a water bath, damages the spirochetes less than thawing more slowly, as at room temperature. (4) At storage temperatures of -12°C and -20°C there is a gradual decrease in virulence over a period of days or weeks, and by the 6th week the infectivity of the material is markedly reduced.

The authors conclude that the optimum conditions for the preservation of spirochetes and probably other microorganisms, in the frozen state are afforded by rapid cooling, storage at -78°C, and rapid thawing. These organisms are severly damaged by storage at temperatures of -20°C and higher, and by slow thawing.

Within the paper the authors discuss other phases of the freezing-thawing problem and make the following interesting

statement: "At the risk of oversimplification, then, it can be said that in the maintenance of microorganisms at low temperatures the injury may arise from two sets of factors. The one, associated with the act of freezing and thawing, and the other, associated with the storage period. It seems probable that under the first set of conditions the damage is done by physical changes in the cell or in the surrounding medium."

3. Gag, R. K.
1939. Preservation of Borrelia duttoni by Freezing. J. of Path. & Bact., 49:587-590. (Dept. of Bacteriology, University of Edinburgh.).

Virulent Borrelia duttoni have been maintained one month in tissue frozen at -78°C; whereas the strain failed to multiply in vitro and did not withstand ordinary desiccation and refrigeration.

4. King, A. G.
1930. Viability of the Organism of Rocky Mountain Spotted Fever when Frozen. J. of Infect. Dis., 46: 279-284. (Dept. of Pathology, Harvard Medical School, Boston, Mass.).

This experiment consisted of removing the brain of a guinea pig at the height of its infection with Rocky Mountain Spotted Fever and setting it away in a sterile test tube at approximately -70°C. At various times some of the material was inoculated into normal guinea pigs to test infectivity. A positive test evolved about the clinical course, demonstration of the causative agent in sections of the testicle of the tested pigs, or the exhibition of specific immunity.

In summary the organism of Rocky Mountain Spotted Fever may be kept alive and virulent in well frozen brain tissue for as long as 321 days. Frozen brain is a superior medium to brain in glycerol in the cold. By combining several infectious brains and using the frozen subdivided parts of the mixture, infectivity may practically be insured. It is possible to keep the strain of Rocky Mountain Spotted Fever alive in the laboratory by successive transfers of infectious brain frozen over a long period of time. The infectiousness of blood well frozen for one month was retained in 85% of 13 cases, with a dosage of 5 cc. After 95 days freezing 7.5 cc of blood proved virulent.

5. Bauer, J. H. and Pickels, E. G.
1940. Apparatus for Freezing and Drying Virus in Large Quantities Under Uniform Conditions. J. Exper. Med. 71:83-88.

This paper presents a method (large scale) for preservation of virus (yellow fever) by desiccation, freezing, etc. This method would probably have value in large scale work.

6. Coggeshall, L. T.
1939. Preservation of Viable Malaria Parasites in the Frozen State. Proc. Soc. Exp. Biol. & Med., 42:499-501. (Laboratories of the International Health Division of the Rockefeller Foundation, New York.).

This paper describes a method for preserving monkey malaria parasites for as long as 70 days. The main features of the method are rapid freezing, storage at -76°C., and rapid thawing. Virulence of the organisms is retained.

7. Manwell, R. D. and Jeffery, G.
1942. Preservation of Avian Malaria Parasites by Low-Temperature Freezing. Proc. Soc. Exp. Biol. & Med., 50:223-224. (Dept. of Zoology, Syracuse, University.).

Seven species of avian malaria plasmodia were successfully preserved for periods up to 90 days by low temperature freezing. Speed of freezing and thawing were the important factors. Temperatures ranging between -55°C and -78°C were used in the experiment.

8. Manwell, R. D.
1943. Low Temperature Freezing of Malaria Parasites. Am. J. Trop. Med., 23:123-131. (Dept. of Zoology, Syracuse, University.)

By freezing infected blood in sugar test tubes in a chest of dry ice and alcohol, at least the blood stages of avian plasmodia and Trypanosoma lewisi have been preserved in a viable state for 212 days.

9. Luyet, B. J. and Tartung, M. D.
1941. Factors in the Revival of Anguillula aceti after its Solidification in Liquid Air. Am. J. of Physiology, 133:368-369. (Dept. of Biology, St. Louis University, St. Louis, Missouri.). (Proceedings of the American Physiological Society).

"It has been reported previously that the vinegar eel, Anguillula aceti, a nematode about 2 mm. long, can be revived after having been immersed in liquid air (-195°C) if its water content has been partially reduced by immersion in 30% ethylene glycol and if the worm is rewarmed at a velocity of the order of 1000 degrees per second. The reason of the necessity of so rapid a rewarming is that an exposure of even a tenth of a second to temperatures immediately below zero allows the formation of ice and is fatal. The present report is concerned with the extent of this zone of dangerous temperatures.

The worms, after immersion in liquid air, were exposed for 5 minutes in an iso-pentane bath maintained at a constant temperature from -50°C to -25°C., then they were rapidly rewarmed. None of the animals exposed to temperatures above -40°C. survived, while out of 80 exposed to temperatures below -43°C, 28 survived. The dangerous zone (that of the formation of ice) for the tissues of Anguillula aceti must, therefore, extend from 0°C to about -40°C; at lower temperatures these tissues can be kept in the vitreous state, at least for 5 minutes."

10. Hansom, B. H.
1914-15. The Destruction of the Vitality of Cysticercus bovis By Freezing. J. Parasitol., 1:5-9. (Bureau of Animal Husbandry, U. S. Department of Agriculture.).

A lapse of 21 days following slaughter is amply sufficient to insure the death of the beef cysticercus, and on the other-hand 14 days is not sufficient.

Experimentation shows that if measly beef carcasses are exposed for 6 days to a temperature not exceeding 15°F. the vitality of the cysticerci will be destroyed and that some may survive in carcasses exposed for 5 days to this temperature, though it is doubtful whether they will retain sufficient vitality to develop in the human host, and finally that a considerable proportion may survive in carcasses exposed to a temperature of 15°F, for 4 days or less.

11. Loosanoff, V. L.
1946. Survival and Mortality of Frozen Oysters (O. virginica.). Anat. Rec., 96:586. (Milford Laboratory, U. S. Fish and Wildlife Service.).

The majority of oysters, if frozen but left undisturbed until they thaw out, will survive; however, if frozen oysters are shaken or subjected to any other type of rough handling, heavy mortality will occur among them. In some of the experiments 100% of the oysters, which were first frozen and then shaken in a basket, died, while almost all of the animals in another basket, which was frozen but not shaken, were found alive upon thawing. Ten weeks after the end of the experiment the thawed oysters were apparently in a healthy condition. The mortality among the shaken oysters was probably due to the damage caused by the rearrangement of ice crystals within the body cells of the mollusks.

These experiments were run during the winter of 1945-46 with the oysters of Long Island Sound and Milford Harbor. The age of the oysters used ranged from 6 months to 5 years.

12, Gaylord, W. R,
1908, The Resistance of Embryonic Epithelium, Transplantable Mouse Cancer, and Certain Organisms to Freezing with Liquid Air, J. Infect. Dis., 5:443-448. (New York State Cancer Laboratory, Buffalo, New York.).

The author notes the findings of J. E, Salvin-Moore, C. E. Walker, and J. O, Wakelin Barrett in studies of the effects of liquid air upon the graftable cancer of mice, These authors found that transplantable mouse cancer can be exposed to the freezing of liquid air for from 20 minutes o half an hour and that such frozen material inoculated into mice is capable in a certain number of instances of producing growing tumors. The tumors which develop from such grafts are essentially of the same histological appearance as the tumors from which they were taken. Although these authors give prominence to the idea that the freezing in all probability destroys the cancer cells, but leaves intact some virus that stimulates the cells of the host to proliferation with the formation of a new tumor, they consider the possibility of the cancer cells being able to withstand this low temperature. Inasmuch as some bacteria and trypanosomes are said to survive this temperature for a period of 20 minutes, and as normal tissue cells are not supposed to be capable of resisting such temperature, they are inclined to believe that these experiments indicate the presence of a parasite in the cancer tissue,

A,

Ca. of mouse (G 1,532 D)	Frozen 40 minutes	9 susceptible mice	2 developed tumors
	Frozen 80 minutes	14 susceptible mice	3 developed tumors
	Unfrozen controls	5 susceptible mice	all developed tumors

B, Determination of Resistance of Trypanosoma gambiense to freezing with Liquid Air,

Spleen of rate with T. gambiense	Frozen 20 Min,	2 rats	both died (virulence somewhat decreased.)
	Frozen 40 Min	2 rats	Trypanosomes destroyed
	Frozen 80 minutes	2 rats	Trypanosomes destroyed
	Unfrozen control	2 rats	Both died,

C. Determination of Resisting Power of Growing Epithelium to freezing.

Aseptic tissue of mouse embryo	Frozen 20 Min.	9 mice	No growth - necrosis of tissue
	Frozen 40 Min.	8 mice	
	Frozen 80 Min.	14 mice	
	Unfrozen Controls	5 mice	Showed growth

"In the case of embryonic tissue, it is a well known fact that epithelium transplanted into subcutaneous tissues of animals of the same species shows distinct evidence of proliferation and growth, frequently for a considerable period of time."

Gaylord concludes: (1) The cells of transplantable mouse cancer can withstand freezing for a period of 80 minutes and still produce tumors. The percentage of inoculations is greatly diminished, the tumor s appear later and grow more slowly than when transplanted directly. They present the same histological picture as the tumors from which they were taken and the controls. (2) Embryonic tissue is killed by freezing with liquid air. (3) Trypanosoma gambiense can resist freezing with liquid air for a period of 20 minutes. It is killed at 40 minutes.

13. Breedis, C.
1942. The Action of Extreme Cold on Leukemic Cells of Mice. J. of Exper. Med., 76:221-240. (Dept. of Pathology, Cornell University Medical College, New York.).
The author makes the observation that viruses, bacteria, and single celled or multicellular animals and plants may survive the temperatures of liquid air or lower and that the resistance of those forms that showed marked cold hardiness is correlated with ability to survive desiccation. fter experimentation with the effects of extreme cold on leukemic cells of mice, the author makes the following notations: (1) Suspensions of leukemic cells rapidly frozen to -196°C were in all cases innocuous; whereas, those frozen slowly were capable of transmitting leukemia. The infectivity of slowly frozen material varied from an estimated 0.0001 percent of that of fresh material, and this figure probably represents the percentage of surviving leukemic cells. (2) Particles of spleen and lymphnode reacted to slow and rapid freezing in the same manner as the suspensions prepared from them. (3) For one of the three strains studied, rapid thawing was less injurious than slow thawing; for the other two strains the rate of thawing seemed to be immaterial.

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(4) Infectivity was equally well preserved after freezing to -21°C whether freezing occurred spontaneously after supercooling or was initiated near the freezing point by inoculation with ice, or whether thawing was rapid or slow. (5) Suspensions already slowly frozen at temperatures of -2°C or lower, whether spontaneously or by inoculation with ice, could no longer be completely inactivated by subsequent rapid cooling to -196°C. Unfrozen suspensions initially above the freezing point or supercooled to -2°C or -8°C and then rapidly cooled to -196°C were inactivated. This protective action of previous slow freezing was most marked when the initial temperature of the frozen suspension was -15°C or lower; when it was -2°C protection was barely detected.

The author states that these observations indicate that the changes which are peculiar to rapid freezing alone and lead to complete inactivation take place during rapid transition from the liquid to the solid state, in a range of temperature lying between -15oC and the freezing point. Temperature measurements carried out in this range showed that suspensions were about equally infectious whether the temperature at their centers dropped from 0°C to -15°C in 30 minutes or in 1 minute; when the drop occurred in 12 seconds or less, the suspensions became inocuous.

It should be noted that in many respects these findings are not in agreement with those on other forms of animal and plant life for rapid freezing usually results in a preservation of such life.

14. Lambert, R. A.
1913. The Influence of Temperature and Fluid Medium on the Survival of Embryonic Tissues in Vitro. J. Exper. Med., 18;406-411. (Dept. of Pathology of Columbia University, College of Physicians and Surgeons, New York.).

In this experiment the author preserved small pieces of chick and rat embryo in hanging drop plasma (Ringers and Physiologic saline) suspensions. The specimens were divided into groups and treated with varying temperatures ranging from -7°C to 20°C. After storage the specimens were transferred to a fresh drop of plasma and incubated at 37°C.

The author concludes: (1) Embryonic chick and rat tissues preserved at temperatures ranging from -7°C to 20°C live longest at about 6°C. The duration of life under the most favorable conditions is less than 20 days. (2) The kind of isotonic medium used (plasma, serum, Ringer's, or normal salt solution) does not appreciably influence the period of survival. The quantity of medium in proportion of tissue

is similarly without marked effect. (3) Those specimens found to be frozen were invariably killed.

15. Hoagland, H. and Pincus, G.
1942. Revival of Mammalian Sperm after Immersion in Liquid Nitrogen. J. Gen. Physiology, 25:337-344. (Physiological Laboratories, Clark University, Worcester.).

In this experiment a wide variety of procedures were used to test the motility of mammalian sperm after plunging them into liquid nitrogen at -195°C and later rapidly warming them to 35°C by plunging them into a suitable balanced and isotonic medium.

On using seminal fluid sperm from the same human donar, the authors found maximal numbers of motile sperm survived vitrification when the samples were very fresh, untreated with plasmolysing solutions, and plunged into the refrigerant in the form of a foam. The maximal yield of motile human sperm was 50%. Since in this sample only 75% of the sperm were alive before immersion, 67% of the living sperm survived vitrification.

Experiments with sperm from 31 rabbits were made under a variety of conditions. Partial drying and plasmolysis gave consistent yields of motile sperm after vitrification by liquid nitrogen.

16. Parkes, A. S.
1945. Preservation of Human Spermatozoa at Low Temperatures. Brit. Med. J., 2:212-213. (National Institute for Medical Research, Hampstead.).

This paper gives considerable review data concerning the preservation of spermatozoa at low temperatures following which the author conducts some experimental work on the subject. His conclusions are as follows: (1) A large proportion of human spermatozoa survive for long periods of time in semen frozen in bulk at -196°C or -79°C. (2) Spermatozoa do not survive when minute amounts of semen are frozen as films or in fine capillary tubes. (3) The rate of freezing and thawing is not the primary factor in the survival of human spermatozoa exposed to low temperatures.

17. Shaffner, C. S.
1942. Longevity of Fowl Spermatozoa in Frozen Condition. Science, 96:337. (Poultry Department, Purdue, University.)

In this paper the author cites evidence of the preservation of life in monocellular organisms by storage at low temperatures. ention is made of a technique for preserving chicken spermatozoa by storage at low temperatures. Results of experiments using slight modifications of the original technique indicate that time is not an important factor in the retention of motility within the first year, when fowl semen is held constantly at the temperature of solid carbon dioxide. Spermatozoa have been maintained at a temperature of dry ice (-79°C) for 14 months. Little, if any, difference could be noted in the percentage of cells that regained motility between samples thawed immediately after freezing or those thawed after 14 months storage.

Unmated hens producing infertile eggs were inseminated with semen that had been frozen at -79°C and thawed an hour later. Of 48 eggs produced by these hens after insemination 12 were fertile; however, in no case did the resulting embryonic development proceed for more than 10 to 15 hours, as determined macroscopically.

18. Shettles, L. B.
1940. The Respiration of Human Spermatozoa and their Response to Various Gases and Low Temperatures. Am. J. Physiol., 128:408-415. (Dept. of Obstetrics, Johns Hopkins University and Hospital.).

To ascertain whether human spermatozoa could be resuscitated after exposure to -79°C., different samples approximately one hour old were placed in a bath at this temperature. A column of undiluted semen was drawn up into thin-walled capillary tubes with an internal diameter of approximately 0.2 mm. After the ends were sealed, the tubes were instantly immersed into alcohol cooled with dry ice. After five minutes at this temperature, the semen was thawed by placing the tubes into a water bath at 37°C. Ten minutes later the semen was examined for active spermatozoa. To observe the effect of much greater depressions of temperature, the preceding experiment was repeated with liquid nitrogen and liquid helium (196°C and -269°C). In order to study the effect of prolonged exposure to low temperatures, specimens of semen one hour old were kept at -79°C for one month and in some instances for more than two months. In these tests, capillary tube preparations were thawed by placing them in a bath at 20° and 37°C.

The spermatozoa resuscitated in these experiments appeared to be in good morphological condition, and those which regained their motility continued active for several hours. After freezing plus drying the morphology was preserved, but there was no resuscitation.

The rate of respiration of human spermatozoa varies inversely with the age of the specimen and directly with the number of cells per unit volume. No oxygen is consumed by semen devoid of spermatozoa. The respiratory quotient of human spermatozoa varies inversely with the age of the specimen and directly with the rate of oxygen consumption, indicating a shift in the metabolites being oxidized. The age of the spermatozoa and the number of cells per unit volume may not be the only factors involved in the consumption of oxygen, for the results indicate that individual variation is also a factor."

19. Brown, E. and Landis, E. M.
1946. The Effect of Cold on Capillary Permeability and Fluid Movement in the Frog. Biol. Bull., 91:236.

Micromanipulative methods were used to study the relationship between capillary blood pressure and the rate of movement through the walls of single capillaries in the frog's mesentery at 22.5 - 25.5°C and between -2 and plus 2°C. Cooling decreased capillary permeability, reduced the observed rates of filtration and increased the observed rates of absorption.

The effects of cold on the capillaries of the frog differ from those observed in mammalian capillaries because the former become less permeable at 2°C, whereas the latter become more permeable as the temperature falls below 10°C. However, actual freezing of the frog's capillaries at temperatures of -5° to -10°C increased the capillary permeability conspicuously, as shown by the appearance of stasis after thawing. If the duration of actual freezing was brief, this stasis usually disappeared within a few minutes as the capillary wall regained its normal relative impermeability to protein.

20. Luyet, B. J. and Thoennes, G.
1938. The Survival of Plant Cells Immersed in Liquid Air. Science, 88:284-285 (Dept. of Biology, St. Louis, University.).

After a review of the literature concerning the survival of plants and animals exposed to extremely low temperatures, the authors conclude that two kinds of organisms can support an immersion in liquid air; first, those which resist previous drying (seeds, spores, protozoan cysts, tardigrades, and

nematodes) and second, those which do not exceed a few micra in size (bacteria, yeast, monocellular algae, and flagellates of the type trypanosome). The authors suggest that their survival might be due to the fact that water does not crystallize in these organisms.

After studying vitrification in gelatin gels the authors make the following conclusions: (1) Gelatin gels containing 37 to 90% water can be brought into the vitreous state by sudden immersion in liquid air. (2) The thickness of the vitrifiable layer decreases with increasing water content, extending from 0.3 mm to a few micra when the water content varies from 50 to 90%. (3) Those temperatures at which the material crystallizes cover a range of some 15 degrees (from 0°C to about -15°C); crystallization takes place either during a cooling from the atmospheric to a sub-zero temperature or during a warming from the lower temperatures to above -15°C. (4) The possibility of obtaining the vitreous state depends primarily on the velocity of crystallization -- pure water could not be vitrified on account of its excessively high crystallization velocity, while gelatin gels are capable of vitrification in proportion to the slowness of their crystallization, that is in proportion to their gelatin concentration. (5) The vitrifying procedure consists essentially in cooling rapidly enough to bring the temperature of the material across the zone of crystallization temperatures before the ice crystals have time to form.

After experimentation with onion epidermis the authors conclude that to save the cells from disintegration one must dehydrate them, cool them rapidly, and finally warm them rapidly. This suggests that the disintegration of protoplasm subjected to extremely low temperatures is due to crystallization and that any method, such as vitrification, which prevents crystallization, prevents protoplasmic disorganization.

DIVISION IV.

SOME ASPECTS OF COLD ON HIGHER ANIMALS.

1. Carrel, A.
1910. Latent Life of Arteries. J. Exper. Med., 12: 460-485. (Laboratories of the Rockefellar Institute for Medical Research, New York.).

"When a segment of artery, killed by heat, formalin or glycerin is transplanted, it undergoes a rapid degeneration. Its muscle fibers disappear while the tissue of the host reacts by building a new wall of connective tissue. When the transplanted vessel has been preserved in a condition of latent life, no degeneration of the wall occurs, or the wall undergoes only partial degeneration. The muscle fibers can keep their normal appearance, even for a long time after the operation. It is, therefore, demonstrated that arteries can be preserved outside of the body in a condition of unmanifested actual life."

"The best method of preservation consists of placing the vessels, immersed in vaseline, in an ice box, the temperature of which is slightly above the freezing point."

2. Carrel, A.
1912. The Preservation of Tissues and Its Application in Surgery. J. A. M. A., 59:523-527.

In this paper the author concludes: "The results obtained by Tuffier, Hagibot and myself demonstrated that human tissues preserved in cold storage could be used in human surgery. Future investigators will show in what measure tissues of infants should be employed in grafts. The grafts could easily be taken in large quantities from the fresh cadavers of fetuses and infants, and preserved in petrolatum and in cold storage. A supply of tissues in latent life would be constantly ready for use and the tubes containing the tissues could even be sent, in small refrigerators of the type of vacuum bottles, to surgeons who would need them. It would simplify very much the transplantation of skin, bone, periosteum and aponeuroses, which are more and more used in surgery."

3. Brooks, B. and Duncan, G. W.
1940. The Effects of Temperature on the Survival of Anemic Tissue. Ann. Surg., 112:130-138. (Dept. of Surgery, Vanderbilt University, Nashville, Tenn.).

These workers applied a constant pressure (130 mm. of Hg.) to produce complete anemia in the tail of rats. After determining the time-pressure factor to develop gangrene, they applied varying temperatures between -5°C and 40°C for

variable periods to determine the effect of cold on survival of the tissue (gangrene being the measure of failure.).

Their results indicate that as the temperature was reduced from 40°C to 1°C the pressure could be maintained longer without the production of gangrene. Using -5°C (freezing) gangrene invariably ensued. As a corellary, however, microscopic study often showed fibrosis in muscle and bone of the tissues which did not become actually gangrenous.

4. Fay, Temple
1940. Observations on Prolonged Human Refrigeration. New York State J. Medicine, 40:1351-1354. (Temple University Medical School.).

In this paper the first observations in general refrigeration in the human being are presented. Human body temperature can be repeatedly and successfully reduced to around 80°F (rectal), and the patient observed for many hours (up to eight days) without subsequent abnormal effects.

In terminal cases of generalized carcinomatosis, death from cerebral edema and cardiac failure was noted in approximately 15 percent during or following generalized refrigeration. Temporary and prolonged periods of relief of pain were noted in almost every instance. Withdrawal of narcotics was therefore possible. No definite regressive changes in undifferentiated cell growth were noted in deep metastatic lesions following generalized refrigeration. Temporary improvement frequently occurred.

5. Smith, L. L. and Fay, T.
1940. Observations on Human Beings with Cancer, Maintained at Reduced Temperatures of 75 to 90°F. Am. J. Clin. Path., 10:1-11 (Temple University School of Medicine, Philadelphia, Penn.).

It has been shown previously by Fay and coworkers that the prolonged local application of temperatures of 40-50°F. to human neoplastic lesions resulted regularly in the relief of pain and in reduction of the size of the tumor.

"The present studies have brought out the fact that patients can be maintained for periods as long as five to eight days at temperature levels in the eighties; that relief of pain can be regularly anticipated for periods ranging from a few days to as long as five months, and that regressive changes in young embryonal cells, particularly in carcinoma takes place as a result, we believe, of lowered physiological

activity, interfering with the metabolism of these cells. These studies indicate that the entire body economy is reduced, that the circulatory rate and blood flow are slowed, and that the liver is in a relatively dormant and inactive state as shown by the low nitrogenous analyses of the blood."

6. Allen, F.
1944. Theoretical and Experimental Aspects of Surgical Refrigeration. Canad. M. A. J., 51:220-226.

This paper is actually a reprint of an address before an assembly of surgeons. "Although cold is instinctively dreaded asinimical to life, many organisms are known to tolerate extremely low temperatures. Examples are the freezing of bacteria in liquid helium at -450°F and of yeasts at -300°F without apparent injury. Concievably, some organisms can be thus preserved in a state of suspended animation so as to be practically immune to the passage of time. Living bacteria are said to have been recovered from inside the trunks of mammoths frozen for thousands of years in the Siberian ice; and there is the Arrhenius speculation of pansperm, according to which the first germs of life may have been carried to this planet through inconcievably vast time and space at the lowest limits of cold."

"The tolerance of higher organisms for reduction of body temperature is known to vary widely. Some fish can survive freezing in a block of ice. Many cold blooded species and the hibernators among warm blodded species can endure long periods of reduction almost to freezing. The non-hibernating species react to cold with vasoconstriction, shivering, and other complicated reactions governed from hypothalamic centers, the purpose of which is to maintain body temperature. The overtaxing of these defenses leads to exhaustion, and even shock, but Fay s owed how these reactions can be minimized by sedation and rapid cooling so as to produce a partial imitation of hibernation even in the highest species, including man. Metabolism, circulation, and other vital functions are not reduced as low as in true hibernation, and the tolerated temperatures are not as low."

"Acute fatality from cessation first of respiration and then of heart action occurs at certain critical temperatures, ranging from 13 to 14°C for the rat to 22°C for the dog. Human beings were shown by Fay to withstand several days of rectal temperature around 24°C or 74°F. Torpor and the slowing of all physiological processes by cold are characteristic of many poikilotherms, such as the amphibia, and of all higher species in natural or artificial hibernation; but another class of

poikilotherms is exemplified by fish such as the trout and salmon, which are intensely active in icy water. Their protoplasm is evidently geared so that their organs and nervous systems function normally at temperatures near freezing, and in such species the blocking of nerve impulses by cold is evidently impossible."

7. Blackwood, [illegible].
1944. Injury from Exposure to Low Temperatures: Pathology. Brit. M. Bull., 2:138-141.

This paper discusses the typical pathologic lesions resulting from immersion foot and frostbite. In immersion foot, cold (10°C to -1.9°C) and prolonged moisture are the principal etiologic features. In frostbite, cold (about -5°C) alone causes crystallization of tissue fluids, rupture of cell walls and death of the affected tissue. An excellent discussion of the typical lesions of these two states is included in the paper.

8. Feild, J. H., and Hall, V. E.
1944. Physiologic Effects of Heat and Cold. Ann. Rev. Physiol. 6:69-94. (Dept. of Physiology, Stanford, University)

This paper is of a general review type which discusses the effects of heat and cold on the human body from the viewpoint of physiology, medicine, and surgery.

9. Allen, F. M.
1945. Broader Aspects of Refrigeration Anesthesia. Current Researches in Anesth. and Analg., 24:51-65.

This is a most interesting review type article which summarizes much of the effect of cold on embryonic tissue, skin graft tissue, and isolated portions of tissue including carcinoma cells. An interesting comparison of the effects of cold on isolated tissue as compared to the effects of cold on portions of tissue still attached to the living organism is made. The following statement seems worthy of inclusion in this review: "A uniform law is perceived governing the behavior of the entire body and its local parts. Reduction of temperature fixes an inexorable time limit for the survival of the body or any part of the body of a warm-blooded species. But within these survival limits, the fall of temperature itself may be less harmful than the unsuccessful struggle to prevent fall of temperature. Just as sedatives and the other techniques of Fay's "artificial hibernation" purposefully facilitates the fall of body temperature and thus prevents the fatal exhaustion which results from overtaxed bodily

defense reactions, so likewise separation from the body by knife or tourniquet abolishes the local mechanisms of break-down of those mechanisms. "Therefore, the local effects which have been universally attributed to cold are not the result of reduced temperature but of the break-down of the defensive function. Corrspondingly, the first signs of injury are functional, and it is obvious that only when this functional derangement reaches a certain extreme degree do organic lesions follow."

10. Crossman, L. W. and Allen, F. M.
1945. Principles of Surgical and Therapeutic Refrigeration. Surg. Clin. N. A., 25:361-370. (Surgical Service of the City Hospital, Welfare Island, New York.).

This is a general discussion type paper which is based upon the wide experience and experimentation of the authors. "All life is conditioned to a comparatively narrow range of temperature, as regards both optimum function and survival. The variation upward is most limited and absolute; even the most thermophilic microorganisms withstand only comparatively slight temperature elevations, and plant and animal protoplasm is killed even more quickly and positively. The range of tolerance for reduced temperature is far wider, and in general the lethal effect is slow and there are more intermediate grades of injury. The formation of ice crystals or any other physical changes occurring at the critical point of cold are by no means as acutely or irreparably fatal as the coagulation of protein or death of enzymes which occur at the critical point of heat. Between the level at which function presumably stops and that at which death or irreversible changes occur, there is a zone of low temperature which is particularly wide for the lower organisms, but even in the highest species it contrasts decidedly with the abrupt limit of heat."

"It appears theoretically probable that life can persist at absolute zero, judging by the intact survival of some primitive organisms at the lowest attainble temperatures, within a few degrees of absolute zero. Spores are generally regarded as most resistant. The active forms of some but by no means all bacteria, protozoa and algae can withstand similar temperatures. It remains to be proved whether time is thus abolished; i.e., whether with absolute chemical standstill there is immortality at absolute zero. For some organisms the time factor evidently remains potent at minimum temperatures, for they withstand liquid hydrogen for minutes, days or weeks but not indefinitely. Some varieties are resistant whether wet or dry, but the vegetative forms of bacteria furnish examples of great resistance in the dry form and death or attenuation by freezing or repeated freezing and thawing in liquid cultures."

"The general rule, sugject to occasional exceptions, is that resistance to low temperature diminishes as animals and plants become more highly organized."

11. Hemingway, A.
1945. Physiologic Effects of Heat and Cold. Ann. Rev. Physiol., 7:163-180. (AAF School of Aviation Medicine, Randolph Field, Texas.).

In frostbite there may be actual freezing to the extent that crystals of ice form in the tissues; in immersion foot there is damage to tissues without actual freezing. Once a tissue has been badly frozen with ice crystal formation taking place that tissue has been irreparably damaged.

12. Horvath, S. M. and Freedman, A.
1947. The Influence of Cold Upon the Efficiency of Man. J. Aviat. Med., 18:158-164. (Armored Medical Research Laboratory, Fort Knox, Kentucky.)

Reaction times to visual stimuli were not altered during continuous exposure of volunteers to a low environmental temperature for periods of 8-14 days.

Dexterity of the fingers and hand strength was markedly diminished by exposure to low ambient temperatures even when the duration of such exposure was for a relatively short period of time.

DIVISION V.

PUBLIC HEALTH ASPECTS OF FROZEN FOODS.

1. Jordan, E. O. and Burrows, W.
1935. The Production of Enterotoxic Substance by Bacteria. J. Infect. Dis., 57:121-128. (Dept. of Hygiene and Bacteriology, University of Chicago.).

This paper generally reviews enterotoxin production by bacteria. The authors discuss the enterotoxic properties of Staphylococci and the difficulty encountered in restoring these properties once they have been lost. Some strains of alpha and beta Streptococci are able to elaborate enterotoxins; the authors note that this property can sometimes be stepped up by rapid transfers on Starch. Certain Proteus strains, grown under suitable conditions (veal infusion agar slants to which has been added 1% sucrose and under an atmosphere of 25% carbon dioxide) are able to produce a toxic substance which causes symptoms of food poisoning in susceptible monkeys. Workers have established at least one strain each of B. coli and A. aerogenes that produce toxic substances when fed to monkeys. From time to time various workers have mentioned Serratia marcescens, Bacillus subtilis, and other slow lactose fermenters as possible etiologic agents in outbreaks of food poisoning. By the use of rapid transfers on starch medium, enterotoxic production has apparently been proved in the base of some Salmonella aertrycke (typhi murium) strains.

2. Hess, H. E.
1941. Quick Frozen Foods. Mil. Surgeon, 89:638-647. (Veterinary Corps., U. S. Army.).

This article presents a good editorial type discussion concerning the effects of ice crystal size on the autolysis of fish and meats. The author concludes that the smaller the size of the ice crystals the less the damage to the meats. Small ice crystals size in frozen meats is obtained by reducing the storage temperatures rapidly to the lower levels of freezing. The author also discusses bacterial contamination found in processing and preparing the substances.

3. Ostrolenk, M. and Welsh, H.
1942. The House Fly as a Vector of Food Poisoning in Food Producing Establishments. Am. J. Pub. Health, 32:487-494. (U.S. Food and Drug Administration, Federal Security Agency, Division of Bacteriology, Washington, D. C.

The housefly (Musca domestica) has been incriminated as a vector of food poisoning bacteria on many occasions. Classical epidemics of food borne infections, such as the typhoid fever epidemics among soldiers during the Boer and Spanish-American wars, and the widespread outbreak of diarrhea in Southend-on-Sea in 1901 were traced to the contamination of food by flies.

Flies are capable of depositing on food by defecation or regurgitation countless numbers of bacteria. Further the bacterial flora deposited is largely determined by the nature of the food on which the flies feed. Flies infected with Salmonella enteriditis which came in contact with sterile pecan meats deposited their pathogens on the nuts within a period of only 15 minutes.

In their experimental work the authors raised their own flies under sterile conditions; then fed them diets containing virulent Salmonella enteriditis taking care to prevent surface contamination of the flies by the pathogens. The infected flies were then studied in various procedures; a summary of the results follows: (1) Flies fed on food infected with Salmonella enteriditis are capable of infecting other flies as well as the food, water, and miscellaneous surfaces with which they come in contact. (2) Salmonella enteriditis apparently survives in the fly for the duration of itslife, approximately 4 weeks. (3) Transfer of Salmonella enteriditis infection from infected flies to mice and retransfer of the infection from infected mice to flies was successfully carried out. (4) Fly eggs planted in mash infected with Salmonella enteriditis resulted in infected maggots, pupae, and adults.

4. DeFelice, D.
1943. Freezing Foods. Indust. and Engin. Chem. (Indust. Ed.).35:26-28, (New York Agriculture Experimental Station, Geneva, New York.).

The paper presents a survey of the frozen food industry which places special emphasis on the problems of quality of product, palatability, storage, and the like. This is not an experimental work.

5. Lythgoe, H. C.
1943. Cold Storage of Food. Indust. and Engin. Chem. (Indust. Ed.). 35:29-38. (Mass. Department of Public Health, Boston, Mass.).

This paper is an interesting editorial type article which deals in the main with existing State legislation concerning the frozen food industry. The problem of the frozen food industry in regard to the public health is reviewed. This is not an experimental work.

6. Pennington, M. E.
1943. Conservation of Perishables by Refrigeration. Indust. and Engin. Chem. (Indust. Ed.), 35:62-66. (M.E. Pennington, 233 Broadway, New York, New York).

This is a general editorial type work on the practical aspects of the frozen food industry and the possible effects of the war. This is not an experimental type article.

7. Berry, J. A.
1946. Bacteriology of Frozen Foods. J. Bact. 51:639. (Western Regional Research Laboratory, U.S. Department of Agriculture, Albany, California.).

There is a need for standardized methods in the bacteriological examination of frozen foods. Factors of importance are the method of sampling, preparation of the sample, the medium, the time and the temperature of incubation. Tests for specific bacteria, as Escherichia coli, are of doubtful value, since this organism, like others dies during freezing storage. A direct microscopic test would seem better as a gauge of the sanitary history than a culture method. Bacteriological standards for frozen foods must be considered, but should be based on knowledge of reasonable procedures.

8. Cathcart, W. H. and Parker, J. J.
1946. Defrosting Frozen Foods by High-Frequency Heat. Food Research, 11;341-344.

By using high-frequency heat, the constantly annoying problem of defrosting frozen foods can be solved. This is worthy of note for institutional use.

9. Adams, H. S.
1946. Refrigeration in a Food Control Program. Am. J. Pub. Health, 36:1007-1011.

This is a general discussion from the viewpoint of the sanitarian on proper storage temperatures for variable foods and the effect of ultra violet light on the freezing storage of foods. Ultra violet light as a bactericidal agent has several limiting factors, including the necessity of a low initial surface contamination.

10. Simpson, J. I.
1946. Frozen Foods for Institutional Use. J. Am. Dietet. A., 22:661-664. (Frozen Food Foundation, Inc.).

This is an editorial type review of some of the problems of the frozen food industry with major emphasis placed on the problems of quality and palatibility.

11. Fitzgerald, G. A.
1947. Are Frozen Foods a Public Health Problem? Am. J. Pub. Health, 37:695-701. (Director, Frozen Food Foundation, Inc., Syracuse, New York.).

This is an excellent review which deals with the flora of frozen foods, the epidemiology of frozen foods, and Industrial control procedures.

The author states: "In summarizing the work reported, it is evident that the bacteriological flora of frozen foods is very extensive, but of great significance is the apparent presence of certain very active acid producing types so that toxigenic or pathogenic types may be entirely inhibited during the spoilage process. This presumption remains to be unequivocally proved. As yet, therefore, one cannot avoid consideration of the implications of Clostridium botulinum, Staphylococcus aureus, the Enterococci and Salmonella and other pathogenic types which may contaminate frozen foods." (Note the foregoing statement applies to frozen fruits and vegetables.)

"The frozen food industry thus far has failed to establish a sanitary code to protect itself and its customers."

"Frozen foods have been remarkably free from suspicion of being health hazards both as to enterotoxic food poisoning and infectious diseases. An acid-producing streptococcus appears to be active during spoilage, souring foods beyond edibility before enterotoxins can be elaborated. However, there is considerable possibility that pathogenic organisms may survive the freezing-storage treatment and retain sufficient viability to cause infectious diseases. These could be a source of infection in frozen foods of the very best quality because the souring would not be present to warn the user. Frozen fruits and vegetables which may be served in raw salads, could very well be the source of infections. These can be controlled only through careful sanitary procedures in factories."

"Laboratory methods of detecting the incidence of toxigenic or pathogenic strains of organisms are presently inadequate, and frozen foods may momentarily be implicated as health hazards. The industry, accordingly, has every reason to support thorough research designed to develop control techniques."

12. Nason, Edith H.
1947. Recent Trends in Frozen Foods. J. A. Dietet. A., 23:318-321. (Professor of Foods and Nutrition, Syracuse University, Syracuse, New York.).

This paper presents an interesting editorial type review of many of the technical problems which face the frozen food industry. The problems of freezing time, storage temperature, proper selection and treatment of food before freezing are discussed. Bacteriological aspects of the problem are discussed at some length, correlating this problem with that of quality product and palatability. The potential problems of food poisoning and spoilage are discussed.

13. Knox, R. and Walker, J.
1947. Bacteriological Investigation of the Washing and Sterilization of Food Containers. J. Hyg. Lond., 45:151-158. (Emergency Public Health Laboratory, Leicester.).

This paper gives strong evidence of the need for thorough washing and steam sterilization of food containers and utensils in large eating establishments.

14. Jordan, E. O., Dack, G. M., and Pollpert, Oram.
1931. Effect of Heat, Storage and Chlorination on Toxicity of Staphylococcus Filtrate. J. Prev. Med., 5:383-386. (Dept. of Hygiene and Bacteriology, University of Chicago.).

The toxic substance present in Staphylococcus filtrates causing gastrointestinal derangement is not completely destroyed by exposure for 30 minutes to the temperature of boiling water. Some diminution in toxic power may, however, possibly be caused by heating, even at temperatures below 100°C. Similarly the toxic quality does not disappear after storage at a low temperature for as long as 67 days, but is perhaps somewhat weakened. Contact for 3 minutes with a rather strong dose of chlorine did not destroy the toxic quality.

15. McCastline, W. B., Thompson, R. and Isaacs, M. L. 1937. A Food Poisoning Outbreak Due to Staphylococcus. J. Bact., 33:50-51. (Columbia University).

These workers report an outbreak of food poisoning in 31 persons in a college dormitory. The source was apparently ice cream custard. Bacteriological examination of the two flavors served showed total bacterial counts of 4 and 10 billion respectively. In each case Staphylococci accounted for 99.5% of the organisms. Two strains were isolated. Cultures identical with the two strains were isolated from two food handlers.

16. Jones, A. H. and Lockhead, A. G. 1939. A Study of Micrococci Surviving in Frozen Pack Vegetables and Their Enterotoxic Properties. Food Research, 4:203-216. (Division of Bacteriology, Science Service, Dept. of Agriculture, Ottawa, Canada.).

In the course of earlier investigations, these authors noted a marked variability in resistance to prolonged freezing by organisms normally found in frozen pack vegetables. It was shown that Micrococci (including Staphylococci) survived freezing much better than other groups so that they comprised a much larger percentage of the organisms in the frozen as compared with the fresh pack. The present study was undertaken to determine whether strains of micrococci isolated from frozen-pack vegetables were of the food poisoning type, and to note whether frozen-pack vegetables provide a suitable medium for their growth and development.

In their investigations of frozen-pack vegetables, five vegetables (asparagus, spinach, peas, beans and corn) were studied. The vegetables were carefully washed and blanched in boiling water and the cooled and held in a freezer at -17.8°C for variable periods of time (up to 30 weeks). Of the colonies selected for study in the various packs, Micrococci formed from 2.5 to 85.8 %.

The investigators next studied the detection of enterotoxic strains which they had isolated from frozen-pack vegetables. Of the 50 strains of Micrococci from frozen-pack vegetables (representing 980 isolations), twelve strains were positive (representing 411 cultures isolated.) All of the vegetable products tested were shown to harbor one or more strains capable of elaborating enterotoxic substance when grown in a semisolid medium in an atmosphere of carbon dioxide and air and tested by the pipette feeding method. All cultures which gave positive reactions with kittens fermented lactose and mannitol sugar broths.

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Results indicate that in both unfrozen and frozen lots, Staphylococci of the food poisoning type may develop rapidly provided the products are held at room temperature. On the other hand, growth is inhibited at the temperature of the electric refrigerator. Staphylococci filtrates containing enterotoxic substance were held in an electric refrigerator at 4°C for 2 months to determine whether this temperature had any effect on its potency. Results from tests with 6 cultures showed that the enterotoxic capacity was impaired little or none by holding at this temperature.

17. Gilcreas, F. W., and Coleman, M. B.
1941. Studies of Rebaking Methods for Cream-filled Pastries. A. J. Pub. Health, 31:956-958. (Division of Laboratories and Research, New York State Dept. of Health, Albany, New York.).

These workers conducted an experiment to determine, (1) the time and temperature of rebaking necessary to render non-viable an enterotoxic strain of Staphylococcus in custard filled eclair shells, and (2) the palatability of rebaked custard-filled eclairs, chocolate cream pie, and Boston cream pie. The inoculum used was a strain of Staphylococcus aureus from an infected wound of a baker which had produced food poisoning and which produced a positive kitten test. Samples were baked for 15, 20, 30, and 40 minutes at 216 - 220°C.

The results obtained are as follows: (1) Bacterial counts showed the presence of from 200,000 to over 3,000,000 bacteria per ml. immediately before the shells were filled. (2) In the filling from eclairs that had been treated for 15 minutes or longer, no viable Staphylococci were demonstrated either immediately after cooling or after they had remained at room temperature overnight. Heating longer than 20 minutes was found impractical as the shells became definitely toasted; heating for 30 minutes produced charring. (3) The rebaking of eclairs, chocolate cream pie, and Boston cream pie for 20 minutes at 216°C did not impair the appearance or palatability of the pastries.

The authors conclude that the adoption of this procedure in bakeries should reduce greatly the incidence of food poisoning induced by the enterotoxins of Staphylococci in custard-filled products.

18. Segalove, M. and Dack, G. M.
1941. Relation of Time and Temperature to Growth and Enterotoxin Production of Staphylococci. Food Research, 6:127-133. (Dept. of Bacteriology and Parasitology, University of Chicago, Chicago, Illinois.).

In this paper the authors tabulate some 18 outbreaks of Staphylococcic food poisoning reported in the literature in which the incubation time and temperature of incubation are indicated.

In their experiment Staphylococcus aureus strain 161 was used. Small Macaca mullata monkeys were used as test animals. Vomiting was considered the indication of a positive testoof interotoxin formation. To determine the rate of multiplication of the test strain at various temperatures, plate counts were made from cultures grown aerobically in broth or under 20% carbon dioxide en semisolid veal infusion agar, with the result that the test strain grows well at 37°C and apparently not at all at ice box temperatures of 4 - 6.7°C.

The results of their experiments are presented in a table; however, the pertinant data is as follows: (1) Cultures incubated at 37°C for 12 and 24 hour periods gave positive reactions when fed to monkeys in 50 cc amounts. (2) Cultures incubated at 20 to 23.5°C for 3 and 7 day periods gave one positive reaction out of a possible 11 when fed to monkeys in 50 cc. amounts. (3) Cultures incubated at less than 20°C gave consistently negative results when fed in 50 cc amounts to monkeys. (4) When filtrates were given to monkeys by intravenous injection (1cc per kilo. of body weight) one positive out of a possible 3 was obtained after incubation at 18°C for a period of 3 days.

19. Cathcart, W. H. and Herz, A.
1942. Staphylococci and Salmonella Control in Foods. III. Effect of Chocolate and Cocoa Fillings on Inhibiting Growth of Staphylococci. Food Research, 7:96-99. (Research Dept., Am. Inst. of Baking, Chicago, Illinois.).

The growth of Staphylococcus aureus was found to be effectively inhibited by fillings made with natural chocolate and natural cocoa, according to a formula presented in this paper. The inhibitory action was assumed to be due, at least partially, to the pH of the filling. The main inhibitory action, however, appeared to be due to a substance or substances contained in the non fat part of the chocolate or cocoa.

20. Jensen, L. B.
1944. Prevention of Bacterial Poisoning by Food Preservation Methods. J. A. Vet. M. A., 104: 63-65. (Research Laboratory, Swift and Company, Chicago, Illinois.).

"Staphylococci must grow at temperatures between 60 - 115°F for a period of 4 to 8 hours in order to produce enterotoxin sufficient to produce gastrointestinal symptoms." "There are indications that quick freezing of foods and holding of these frozen foods (fish, meat, poultry, eggnagma, etc.) at specific ranges below 30°F may effect a substantial reduction of various bacteria. In storage of natural ice, we have shown that most bacteria die off rapidly, and natural ice stored for a few months is safe even though the original water contained intestinal pathogens. Colon bacilli in ice are killed most rapidly between 30 and 20°F, whereas, at -100°F, destruction is very slow according to Haines."

21. Gross, C. E. and Vinton, C.
1947. Thermal Death Time of a Strain of Staphylococcus in Meat. Food Research, 12:188-202.

The principle significance of this article in this review is that the authors point out the relative heat resistance of Staphylococci among the nonsporeforming bacteria. (Note: Apparently the range of viability for Staphylococci is quite wide.).

22. Bernstein, H. S. and Fish, E. S.
1916. Food Poisoning by the Bacillus Paratyphosus B. An Epidemic Due to the Organism Isolated From Pie. J. A. M. A., 66:167-171.

These authors report a food poisoning epidemic in July 1915 in Westerly, Rhode Island and neighboring townships of Connecticut in which some 60 persons were infected, 4 of whom died. Symptomatology in this outbreak included epigastric pain, burning from the fauces to the stomach, thirst, spasmodic contractions of the calves of the legs, vomiting, diarrhea and tetanus, high temperature and mental depression. The etiologic agent is reported as Bacillus paratyphosus B. The vehicle is reported as pie.

23. Sewell, E. P. Smith, E. B., and Priestley, A. H.
1920. An Outbreak of Food Poisoning in a General Hospital. J. Roy. Army Med. Corps, 34:510-528 (No. 31 General Hospital, Port Said, Egypt.).

This paper reports a violent outbreak of food poisoning in Port Said, Egypt in January of 1917. Some 473 men developed symptoms of whom 3 died. Symptomatology included shivering, headache, generalized aches, cramps, diarrhea, and vomiting. In the most severe cases, dehydration and toxic symptoms followed. Bacillus suipestifer A was isolated from many of the cases. The epidemic was traced to tapioca pudding and tea (which contained milk). The milk was the probable source of the infection. The milk was a powdered product.

24. Salthe, O. and Krumwiede, C.
1924. Studies on the Paratyphoid-Enteriditis Group. VIII. An Epidemic of Food Infection Due to a Paratyphoid Bacillus of Rodent Origin. Am. J. Hyg., 4: 23-32 (Bureau of Foods and Drugs, and the Bureau of Laboratories, Dept. of Health, New York City.).

The authors report an outbreak of 53 cases of "food poisoning" in April, 1943 resulting from the consumption of cream-filled crumb cake and eclairs (the filling was contaminated). The involved bakery was high class, clean, orderly and free of flies and vermin. The bakery personnel were all negative on examination. The offending organism was proved to be Bacillus pestis caviae; the organism was isolated from rat droppings in the shop. None of the cases were very severe and no cases were reported.

25. Gill, D.
1924. Fatal Food Poisoning Due to Bacillus suipestifer B. Brit. Med. J., 2:857.

This paper reports a fatal case of food infection with Bacillus suipestifer (aertrycke) in a 43 year old man which was associated with a drinking bout. The organism was isolated from heart blood, gallbladder, spleen, large and small intestine at autopsy.

26. Damon, S. R. and Leiter, L. W.
1927. The Possibility of Human Infection and Intoxication by Certain Organisms of the Salmonella Group. 1. Rate and Extent of Growth and Physical Changes Produced in Goods by B. suipestifer, B. pestis caviae, B. sanguinarium, and B. anatum. Am. J. Hyg., 7:27-69. (Dept. of Bacteriology of of the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.).

As a result of their studies, the authors make the following self explanatory conclusions: (1) Bacillus suipestifer, Bacillus pestis caviae, Bacillus sanguinarium and Bacillus anatum have been shown to multiply readily in all types of foods except those which have a strong initial acidity. (2) These organisms grow readily in foods at body temperature and at room temperature. (3) Growth of these organisms is but slightly retarded at ice box temperature. (4) Multiplication of these organisms in food under any conditions offers such slight evidence of infection as to be readily overlooked. (5) Prevention of food poisoning by these organisms must therefore, depend upon selection of raw products of unquestioned sanitary quality, care in the process of preparation, so as to obviate contamination, and finally, preservation under such conditions as will assure a minimum of growth in case the food has been contaminated.

The organisms studied were grown in various hosehold foods for periods of 24, 48, and 72 hours at body temperature, room temperature, and ice box temperature (10-15°C). The increase in colony counts at 10-15°C is somewhat amazing.

27. Beard, Paul J. and Cleary, J. P.

1932 The Importance of Temperature on the Survival Time of Bacteria in Acid Foods. J. Prev. Med., 6: 141-144. (Dept. of Bacteriology and Experimental Pathology, Stanford University.).

After studies on the intestinal group of organisms, these authors conclude that: "Decrease in temperature allows the survival of organisms of the intestinal group over dangerous periods of time, at hydrogen-ion concentrations rapidly lethal at ordinary temperatures."

The authors' work is well summarized in their three tables, which are reproduced as follows:

TABLE I.

SURVIVAL TIME OF E. TYPHOSA IN BROTH AT VARIOUS TEMPERATURES AND HYDROGEN-ION CONCENTRATIONS.

Strain	pH	Hours of Survival at 37.5°C	-4°C	-12°C*
Rawling	4.0	50	96	135
Kuttner	4.0	50	150	120
Rawling	3.6	24	168	108
Kuttner	3.5	24	144	108

*Frozen

TABLE 2.

SURVIVAL OF ORGANISMS IN BROTH AT -12°C.

Organism	Hours of Survival at pH 4.0	pH 3.5
Salmonella enteriditis	80	72
Salmonella schottmulleri	120	60
Salmonella schottmulleri	120	72
Staphylococcus aureus	45	40
Staphylococcus albus	45	40

TABLE 3.

SURVIVAL OF ORGANISMS IN ORANGE JUICE

Organism	Hours of Survival at -4°C., pH 3.5
Eberthella typhosa (Rawling)	170
Eberthella typhosa (Kuttner)	170
Shigella dysenteriae (Shiga)	170
Salmonella schottmulleri	72

The authors state that this date suggests the possibility of infection by this group of organisms in highly acid foods preserved only by storage at such temperatures. This possibility of infection is increased by the fact that such foods are frequently served with little storage - indeed freshness is a selling point."

Continued.

28. Roberts, J. and Wilson, R. J.
1939. A Third Outbreak of Staphylococcal Food Poisoning in Hamilton, Ontario. Canad. Pub. Health J., 30:590-598.

In this outbreak of food poisoning some 47 households were invaded. The victims suffered abdominal pain, vomiting and diarrhea which lasted from 1½ to 35 hours. Every victim attended a dinner at a local church. The menu consisted of cold roast pork, applesauce, beans, potatoes and gravy, coconut cream pie, coffee, and cream. Staphylococcus aureus was isolated from the cream pies. Study of the bakery in which the pies were produced revealed that the pies were consumed approximately 24 hours after manufacture and that the incubation temperature was room temperature. An enterotoxin was elaborated by the strain of organism convicted as the etiologic agent. Five of the eight bakery workers had positive cultures for Staphylococcus aureus on nose and throat culturing. Several families outside of the church circle were also infected by pies from the same lot.

29. McCleskey, C. S. and Christopher, W. N.
1941. Some Factors Influencing Survival of Pathogenic Bacteria in Cold-Pack Strawberries. Food Research, 6:327-333. (Louisiana State University, Baton Rouge, Louisiana.).

In this paper rather extensive inoculation experiments are reported. Certain pathogenic bacteria were inoculated into commercial-pack strawberries, frozen in about 2½ hours and then stored at -18°C The authors summarize their work as follows: "Certain pathogenic bacteria inoculated into sliced sweetened strawberries and held at -18°C were recovered after varying periods of storage as follows: Eberthella typhosa, six months; Salmonella Aertrycke and Salmonella schottmuelleri, one month; Staphylococcus aureus, five months; Salmonella paratyphi was not recovered at any time from the frozen berries". "Eberthella typhosa inoculated into unsliced but sweetened berries were still present in small numbers after 14 months storage at -18°C". "The use of 50% sucrose as the dilution water for making plate counts of Eberthella typhosa in frozen-pack strawberries made it possible to obtain satisfactory quantitative data. Ordinary dilution water and tryptone broth were not satisfactory for making dilutions." "The death rate of Eberthella typhosa in strawberries held at room temperature was very rapid, such that heavily inoculated berries were free of living germs after six hours. Held at 5°C the deathh rate was such that about 98% were killed in one day, and sterility was reached in eight days.

Continued.

30. Stone, W. S.
1943. Food Handlers in the Army and Their Relationship to Salmonella Food Poisoning. Am. J. Pub. Health., 33:706-708. (Office of the Surgeon General, Preventive Medicine Service, Washington, D. C.).

In this paper the author points out that in war time and during periods of congestion of population, there is greater chance for carriers (food handlers) to transmit their disease. In reviewing the Panama Canal Department, the author states that small outbreaks of food poisoning, diarrhea, and dysentery in various commands prior to 1940. In May of 1940 a careful laboratory examination was undertaken; in the following six months some 12 food poisoning outbreaks were studied, in 11 of which various species of Salmonella were isolated and further a direct correlation between food handler carriers and the epidemics was noted. In the course of the studies some 2,000 individuals were studied; of these 49 proved to be carriers of intestinal pathogens.

The distribution of intestinal pathogens discovered is as follows:-

1.	S. typhi	4	9.	S. oranienburg	3
2.	Shigella (Sonne)	3	10.	S. montevido	1
3.	Shigella (Flexner)	2	11.	S. newport	6
4.	S. paratyphi B	2	12.	S. panama	3
5.	S. paratyphi B var. Java	12	13.	S. give	1
6.	S. typhimurium	5	14.	S. anatum	1
7.	S. derby	2	15.	S. arechaveleta	1
8.	S. paratyphi C	2	16.	S. saint paul	1

31. Stuart, C. A. and Rustigan, R.
1943. Further Studies on One Type of Paracolon Organism. Am. J. Pub. Health, 33:1323-1325. (Biological Laboratory, Brown University, Providence, Rhode Island).

"For many years continental bacteriologists, including the English have considered the paracolon organisms as a group intermediate between the coliform and Salmonella bacteria." These workers are of the opinion that some members of this group are pathogenic, some questionably pathogenic, and some definitely non-pathogenic. After considerable previous experimental work, the authors feel that this group could be divided into three sections: paracolon Aerobacter, Paracolon intermediates, and paracolon Escheriohia according to their IMVIC pattern.

The authors are of the opinion that biochemical type 32011 Aerobacter offers the best evidence for pathogenicity and state that patients listed as "typhoid suspects" sometimes yield this strain of the organism. This biotype frements

glucose, maltose, mannitol, and frequently salicin in 24 hours. Lactose and sucrose are fermented slowly or not at all.

32. Galton, M. M. and Guan, M.S.
1944. Salmonella Isolated in Florida during 1943 with the Combined Enrichment Method of Kauffman. Am. J. Pub. Health, 34:1071-1075. (Bacteriologist, Bureau of Laboratories, Florida State Board of Health, Jacksonville, Florida.).

In this paper the authors state that the combined enrichment method of Kauffmann has proved to be excellent for the isolation of Salmonella from routine feces specimens. They attribute their 164 % increase in positive Salmonella finding to the efficacy of the medium. The method did not prove as favorable for the isolation of typhoid and Shigella.

The authors have continued to find in Florida a wide variety of Salmonella types and believe that there occurrence is due in part to the large numbers of transient persons entering and leaving the state.

33. Edward, P. R. and Hughes, H.
1944. A New Salmonella Type with Hitherto Undescribed Somatic Antigens. Proc. Soc. Exper. Biol. and Med. 56:33.

Salmonella inverness, a new Salmonella type isolated from a normal food handler is described. This organism possessed a hitherto undescribed somatic antigen and was assigned the antigenic formula: XXXVIII - k - 1,6 ....

34. Schneider, M. D.
1945. Isolation of Salmonella tennessee from Frozen Whole and Powdered Egg. Bull. U. S. Army Med. Dept., 4:477. (Veterinary Corps, Army of the United States.).

Four cultures of S. tennessee were isolated from both frozen whole and powdered egg with a moisture content not exceeding 2%. The evidence established Iowa and Nebraska as sources of these organisms which so far as is known have not been previously reported in the midwestern states. The implication is that fowl may, as in the case of many other salmonellae, be the source of this type Salmonella.

35. Garrard, E. H.
1946. Coliform Contamination of Eggs. Canad. J. Research, C. 24:121-125.

Bacteriological examination of 1080 eggs from pullorum infected hens showed 78 (7.2%) to be contaminated with coliform organisms. No coliform organisms were isolated from 1000 eggs laid by hens free from pullorum disease.

36. Stuart, C. A., Wheeler, K. M., and McGann, V.
1946. Further Studies on one Anaerogenic Paracolon Organism, Type 29911. J. Bact., 52:431-438. (Biological Laboratory, Brown University, Providence, Rhode Island and the Bureau of Laboratories, Connecticut State Dept. of Health, Hartford, Conn.).

Type 29911 cultures present a problem in taxonomy. Included in the group are cultures previously designated as an anaerogenic paracolon type (Stuart, Wheeler, et al., 1943), as B. wakefield related to the Flexner dysentery organism, as mannitol-negative types of Shigella, as Sachs types B81 and B105. The authors state that the present study indicates that type 29911 closely resembles Proteus species in their biochemical and IMVIC reactions, their ability to swarm when properly conditioned, their reaction to urea, and their many minor and occasional major antigens in common with Proteus would call for an extension of the limitations of the genus which in the authors opinion would not be warranted at this time. Apparently type 29911 occupies a position intermediate between the Proteus and Shigella groups.

Considerable evidence has accumulated to show that type 29911 organisms cause gastroenteritis and diarrhea. Aside from their own work the authors cite the work of Berger, Sachs, and Galton.

Experimental studies with 109 cultures of anaerogenic paracolon type 29911 and related cultures revealed the cultures to be biochemically and serologically heterogeneous.

37. Straka, R. P. and James, L. H.
1932. A Health Aspect of Frozen Vegetables. Am. J. Pub. Health, 22:473-492. (Food Research Division, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D. C.).

This work is a study of tinned and carton packed frozen peas which were inoculated with Clostridium botulinum spores prior to packing. Results which seem pertinant to this review follow: (A) "Examination of 24 tin containers of uninoculated peas, most of which were held for 3 days after defosting, showed 2 to be toxic. Eight others yielded

botulinus cultures, 2 of which were obtained from samples examined immediately after being defrosted. (B) Examination of 24 tin containers of peas lightly inoculated, most of which were held for 3 days after defrosting, revealed 4 to be toxic. (C) Of 24 tin containers which had received a heavy inoculation, most of which were held for 3 days after defrosting, 8 were toxic. (D) Of 16 cardboard containers which had received no inoculation and most of which had been held for 3 days after defrosting, 2 were toxic, and 3 others yielded botulinus cultures. (E) Of 16 cardboard containers which had received a slight inoculation and most of which were held for 3 days after defrosting, 2 were toxic. (F) Of 16 cardboard containers which had received a heavy inoculation and most of which were held for 3 days after defrosting, 5 were toxic. (G) No toxin developed in peas which were examined immediately after defrosting, and none developed in those defrosted and held for 3 days in the icebox. (H) The contents of all the containers which showed toxin had been stored at room temperature and were badly spoiled when examined.

38. Straka, R. P. and James, L. H.
1933. Frozen Vegetables. Am. J. Pub. Health, 23: 700-703. (Food Research Division, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D.C.).

This paper presents a study of Clostridium botulinum toxin production in inoculated hand shelled peas which had been frozen in 16 ounce commercial glass jars. Experimentation revealed that toxin was obtained only in the defrosted samples of peas that were held at room temperature. All samples containing toxin were definitely spoiled. No toxin was discovered in containers defrosted and examined immediately or in containers defrosted and held in an ordinary icebox.

39. James, Lawrence H.
1933. Effects of Freezing on The Spores and Toxins of Clostridium botulinum. J. of Inf. Dis., 52:236-241. (Food Research Division, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D. C.).

As spores of microorganisms are less affected by freezing than are vegetative cells, it is important to consider the possibility of development of botulinus toxin in improperly handled defrosted frozen pack fruits and vegetables. This paper presents experimental data on the effects of freezing, and thawing on the spores and toxin of Clostridium botulinum, type B first in Sorenson's buffer solution (pH 6.9) and second in pea juice (pH7.1). The suspensions were frozen by packing in solid carbon dioxide and then grown in beef-heart meat tubes overlain with autoclaved petrolatum for

for not less than 21 days. Tubes with gas production and digestion of meat were retested and toxin was demonstrated byiinocculation of guinea pigs. The results indicated that few if any spores of Clostridium botulinum (as encountered in soil or vegatables) are killed by quick or slow freezing. In another portion of the experiment spores of Clostridium botulinum, type B were suspended in pea juice and were frozen by means of solid carbon dioxide and defrosted repeatedly. The results indicate that freezing and thawing will break up clumps of bacterial spores and will result in slight destructive activity on the spores; however, in the presence of millions of spores, the small reduction noted is comparatively unimportant. In a third portion of the experiment, supernatant liquor of a centrifuged toxic broth culture was frozen both in solid carbon dioxide and in a refrigerated room. After the fourth freezing there was evidence of but slight reduction in the amount of toxin present. Inaa fourth portion of the experiment, botulinus toxin frozen and defrosted. fifteen times was found not reduced in strength.

40. Straka, R. P. and James, L. H.
1935. Further Studies on Frozen Vegetables. J. Bact. 29:313-322. (Food Research Division, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington D. C.).

This research was undertaken to study the possibility of toxin production in frozen peas when defrosted and held for a shorter period than 3½ days at room temperature, and when held for al longer period at icebox temperature. A total off 198 samples of frozen peas in various types of containers were examined. The results of the experiment are as follows: (1) Of 83 uninoculated controls (33 defrosted and held at 80°F for 2 days, 20 defrosted and held at 60°F for 3 days, and 30 defrosted and held at 50°F for 7 days), only 1 gave positive evidence of a weak toxin. (2) Of 15 containers receiving a dilute inoculum (defrosted and held at 80°F for 2 days), none gave evidence of toxin. (3) Of 100 containers receiving a concentrated inoculum (30 defrosted and held at 80°F for 2 days, 15 defrosted and held at 60°F for 3 days, 30 defrosted and held at 50°F for 7 days, and 10 defrosted and held at 42°F for 7 days), nine gave definite evidence of toxin (6 had been held at 80°F and 3 had been held at 50°F.).

The authors conclude: "When peas preserved by freezing are properly handled there is no danger of botulism. They should not be held at room temperature after defrosting, and left-over portions should be well refrigerated and thoroughly cooked before consumption.".

41. Prescott, S. C. and Tanner, F. W.
1938. Microbiology in Relation to Food Preservation. Food Research, 3:189-197. (Dept. of Bacteriology, University of Illinois, Urbana.).

This is an interesting review type paper based upon the literature and the extensive experience of the authors in this field of endeavor. The effects of freezing on pathogenic bacteria with special emphasis on the effect of freezing on bacteria in foods is discussed.

DIVISION VI.

MICROBIOLOGY OF FROZEN FOODS.

1. Prescott, S. C. and Bates, P. K.
1931. The Reduction of the Number of Organisms in Water as a Result of Freezing in Domestic Refrigerators. J. Bact., 21:26. (Mass. Institute of Technology, Boston).

All observations indicate freezing reduces the number of organisms in water. Very small numbers of organisms persist for some days, but there is no appreciable increase in organisms over periods up to eleven days.

2. Prescott, S. C., Bates, P. K., and Needle, H. C.
1931. The Effect of Discontinuous Refrigeration on Bacteria in Foods. J. Bact., 21:25-26, (Massachusetts Institute of Technology and Bacteriology Department, Frigidaire Corp.).

Foods which are refrigerated discontinuously show increases in bacterial growth; this increase is especially rapid at higher temperatures. Spoilage of foods takes place much more quickly in discontinuously refrigerated foods, and especially when the storage dishes are covered. Occasionally growth increased rapidly even at the lower temperatures for those foods discontinuously refrigerated.

3. Prescott, S. C. and Bates, P. K.
1931. On the Relation of Refrigeration Temperatures to the Rate of Growth of Certain Specific Type s of Bacteria Causing Food Spoilage. J. Bact., 21:25. (Massachusetts Institute of Technology and Bacteriology Dept., Frigidaire Corp.).

It was found that the different organisms of the same general class frequently have a highly specific action in relation to temperature, and also that at lower temperatures, the relative rates of growth in pure culture are proportional to the temperature increments only for brief periods if at all. Perhaps the most important finding is that certain types of spoilage organisms adapt themselves to temperatures assumed to be inhibitive to d ecomposition processes.

4. Castell, C. H., and McDermott, L. A.
1942. Multiplication of Bacteria in Water and its significance in Food Spoilage. Food Research., 7: 244-253. (Dept. of Bacteriology, Ontario Agricultural College, Guelph, Canada.).

"The following species showed active multiplication in the water, reaching counts of over 500,000 per milliliter: Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas

fragi, Aerobacter aerogenes, Serratia marcescens, and Achromobacter lipolyticum. Pseudomonas putrefaciens gave variable results, showing in one instance counts of from 200 to 73,000 per milliliter after 160 hours, but it apparently grew better in water when accompanied by certain other organisms."

"Escherichia coli, Proteus vulgaris, Alcaligenes viscosus, Staphylococcus aureus, Staphylococcus citreus, Sarcina lutea, Micrococcus conglomeratus, Bacillus subtilis, Bacillus mycoides, Bacillus graveolens, Bacillus panis, and Bacillus mesentericus showed no significant increases over a period of 20 days."

5. Gorseline, H. E.
1946. Microbiologic Examination of Foods: Tentative Methods for Microbiological Examination of Frozen Foods. Report of the Committee on Standard Methods for the Microbiological Examination of Foods. Am. J. Pub. Health 36:332-335.

This is an especially pertinant paper with regard to bacteriological methods and should be referred to by any worker approaching this field. Some of the broader aspects of the paper may be summarized as follows: (1) In testing frozen vegetables for microbiological content, consideration must be given to the fact that freezing storage at temperatures of approximately -10°F (-23°C) brings about a reduction in viable microorganisms. This may amount to roughly 50% in 6 months or 75% in 1 year. Thus, while a high bacterial plate count on frozen vegetables may be taken as proof of poor sanitary history, a low or moderate count does not by itself prove the opposite. (2) For frozen fruits and vegetables, use a mechanical blender in sampling. Tryptone glucose extract agar has been proved superior to Nutrient agar or Glucose agar for fruits and vegetables. (3) An approved method for selection, transportation, storage, preparation, and bacteriologic testing of sample frozen fruits and vegetables is given.

6. Berry, J. A.
1933. Lactobacilli in Frozen Pack Peas. Science, 77: 350-351. (U. S. Bureau of Plant Industry, Seattle, Washington.).

Microbiological analyses of frozen pack peas held in storage from 1 to 26 months show that lactic acid bacteria will tolerate cold storage temperatures. The peas were prepared and stored in a variety of ways; however, the storage temperature in most cases was 15°F, although some packs were held at -5°F.

Some 40 samples were studied for spoilage alone. These were held at ordinary room temperature for from 2 to 7 days, at the end of which time they were analyzed. Without exception the peas appeared bleached and presented a sour odor. The clouded liquors gave positive ferric chloride tests for lactic acid. The pH values ranged between 4.2 and 4.6. Bacteriological analysis yielded Aerobacter aerogenes (colon type) andlactobacilli. Study of 15 cultures of the lactobacilli isolated placed the majority with the Lactobacillus cucumeris type. Inoculation of pure cultures of these Lactobacilli into sterile pees yielded products entirely similar to the original frozen pack peas.

Organisms of this type are widely distributed and occur commonly on vegetables, and their presence on shelled peas is to be expected. Since they are not spore formers, it is noteworthy that they withstand a temperature of 15°F for over 2 years.

7. Berry, J. A.
1933. The Destruction and Survival of Microorganisms in Frozen Pack Foods. J. Bact., 26:459-470. (Frozen Pack Laboratories, U. S. Bureau of Plant Industry, Seattle, Washington.).

The experimental work presented in this paper is concerned with the fate of microorganisms in berries and vegetables held under different conditions of oxygen environment and low temperature storage, for periods varying from 14 weeks to 2 years. The study temperatures were -20°C, -10°C, -7°C, -4°C and -2°C. The berries were packed in sucrose solution; the vegetables in sodium chloride solution.

(1) Data presented shows a decrease of approximately 40% of microorganisms on blackberries stored in airtight and non-airtight containers for 13 months at -20°C; of 99% at -10°C, and in airtight containers only, of over 99% at -2°C. (2) With Strawberries the recorded decrease is 60% in both airtight and non-airtight containers stored for 4 months at -20°C, of 89% at -10°C and -7°C, and in airtight containers only of 94% at -4°C. (3) With raspberries the recorded decrease is 61% when stored at -20°C for 14 weeks, of 95 to 97% when stored at -10°C (4) No significant microbiological difference have been noted between sealed containers and partly vacuumized containers stored at the same temperature.

Death rates in various artificial media at freezing temperatures indicate that they are not comparable to those when the medium is fresh fruit.

Growth of Cladosporium sp. at -2°C and of Oidium sp. and Torula sp. at -4° on small fruit in non-airtight containers has been recorded.

Evidence is presented that Lactobacilli and to a lesser extent "colon" organisms persist in peas for at least 2 years at -10°C. Bacteria, presumably of the Pseudomonas genus, will increase in peas stored at -4°. "Colon" organisms will persist in string beans and spinach at -10°C for at least 19 months and 10 months respectively.

The author compares Saccharomyces sp. in a supercooled state and in a frozen state (both at -10°C) and shows the destructive effect of ice formation on the test organism.

8. Berry, J. A.
1934. Growth of Yeast Below Zero. Science, 80:341. (Frozen Pack Laboratories, U. S. Dept. of Agriculture, Seattle, Washington.).

Though the growth of a "false" yeast (as Torula) below 0°C has been recorded, growth of a "true" yeast has not. This worker reports the growth of a true yeast at -2.2°C in fresh cider. This yeast was not a psychrophile as it grows best at 21°C. Beer wort, pH 4.8, was used as the culture and isolation medium.

9. Shrader, J. H. and Johnson, A. H.
1934. Freezing Orange Juice. Industrial and Engineering Chemistry, 26:869-874. (National Dairy Products Corporation, Inc., Baltimore, Maryland.).

"That the danger of spreading disease through orange juice is negligible, is generally known, but it was considered desirable to study the vitality and growth of Escherichia coli in orange juice because this organism was the best index organism whose reaction would be a dependable guide as to what pathogenic bacteria would do. Cultures of Lactobacillus acidophilus and a member of the Bacillus subtilis group were also included. These three organisms were unable to multiply in orange juice at any temperature; the death rate of the three organisms was most rapid at 37°C, was slower at 25°C and was still slower at -12°C. It was concluded that organisms of the coli type would fail to survive longer than 2 weeks in frozen orange juice but that spores would probably remain for a long time in frozen orange juice. Although

certain strains of yeasts will grow slowly at 0°F, experience in the commercial pack showed that, when frozen juice was stored at about 5°F the yeast, in general, died offf to the extent that at the end of about 6 months the counts dropped from several thousand down to several hundred. Total mixed bacteria counts on standard media likewise decreased on 6 month storage at 5°F."

10. Smart, H. F.
1935. Growth and Survival of Microorganisms at Subfreezing Temperatures. Science, 82:525. (Bureau of Plant Industy, U. S. Dept. of Agriculture.).

"In July, 1933, during a routine examination of frozen strawberries, raspberries, and cherries packed in Oregon in June, 1930, it was noted that while the number of viable microorganisms to be found in the fruit, after 3 years storage at 15°F, was so small as to be practically negligible, there were an unusually large number of interesting species to be seen on the plates. These organisms, representing many species of bacteria, yeasts, and molds, were studied in pure culture and later identified as closely as possible with known species." Some 26 species were transferred to freshly prepared beef infusion agar, pH7.0, slants and stored at 16°F for 1 year. Eight species were able to produce growth at this temperature, 13 species, while showing no signs of growth, did produce abundant growth when removed to room temperature for 24 hours. Only 5 species failed to survive the 1 year storage period on the artificial medium. The author concludes that many species of microorganisms have remarkable faculties for survival as well as for adapting themselves to changes in environment and that this factor must be taken as a warning against careless methods in the preparation of frozen foods.

11. Lochhead, A. G. and Jones, A. H.
1938. Types of Bacteria Surviving in Frozen-Pack Vegetables. Food Research, 3:299-306. (Division of Bacteriology, Dominion Experimental Farms, Ottawa, Canada.).

After extensive studies on fresh and frozen pack asparagus, spinach, peas, beans, and corn, the authors conclude: "In confirmation of previous studies, micrococci and species of Flavobacterium were found to be relatively more resistant to freezing than other types of bacteria encountered in Frozen vegetables. Micrococci in particular comprised a much larger percentage of the organisms in the frozen products as compared with the freshly packed products."

There was not definite evidence that spore-forming rods were proportionately more numerous in frozen than in freshly packed vegetables.

The findings direct attention to the possible importance of the Micrococcus group of organisms in relation to the proper handling of frozen vegetables.

12. Smart, H. F.
1939. Further Studies on the Behavior of Microorganisms in Frozen Cultivated Blueberries. Food Research, 4:287-292. (Food Research Division, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D. C.).

The author summarizes this work as follows: "It has been shown that a high microbial content of frozen blueberries may be an indication of inefficient methods of washing the raw fruit. Freezing and storage of blueberries for 9 months at 0°F resulted in a reduction of the microbial content amounting to 59.7 %, while storage at 20°F for the same period of time resulted in 99.9 % reduction. Quality was preserved in blueberries when they were frozen and stored at 20°F for this period of time. Holding blueberries, immersed in syrup for 24 hours at 45°F before freezing them resulted in a high microbial content as compared with those placed in freezing storage as soon as they were packed."

13. Smart, H. F.
1939. Microbiological Studies on Commercial Packs of Frozen Fruits and Vegetables. Food Research, 4:293-298. (Food Research Division, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D. C.).

Comparison of the microbial content of commercially frozen fruits and vegetables over a period of years indicates that extremely high counts in the products were less common in the 1935 packs than in previous years. The organisms most frequently isolated from these commercially packed frozen foods were the common soil types, which have been considered without health significance but which will cause spoilage of the foods if they are not used promptly after defrosting. Defrosted, commercially packed vegetables were found to spoil much faster than similarly treated fruits when both were held at 30°C for 24 hours.

Microorganisms which the author has repeatedly isolated from commercially frozen fruits and vegetables are as follows: (1) Fruits - - Achromobacter dutyri, Bacillus mycoides, Bacillus aterrimus, Pseudomonas syncyanea, Spirillum volutans, Saccharoyces unisporus, Saccharomyces oxiguus, Aspergillus sp., Mucor sp., Odium sp.penicillium sp,

and Rhizopus sp. (2) Vegetables - - Achromobacter Pellucidum, Bacillus mycoides, Flavobacterium aureseens, Lactobacillus sp., Pseudomonas fluorescens, Sarcina sp., Sarcina subflava, Torula sp., Dematium sp., Penicillium sp., and Rhizopus sp.

14. Pennington, M. E.
1908. Bacterial Growth and Chemical Changes in Milk Held at Low Temperatures. J. Biol. Chem., 4:353-393 (U. S. Dept. of Agriculture, Bureau of Chemistry.).

Bacteria in milk increase in numbers when the temperature is maintained at or a little below 0°C. This temperature is below that ordinarily assigned as the lower limit of bacterial multiplication.

The author stored milk (of variable cleanliness) at temperatures of 29 to 31°F and at 32°F for periods ranging from a few days up to almost 2 years. Bacterial growth at the end of a week (even in the cleanest milk - - 300 organisms per ml.) was pronounced. There was a steady increase in bacterial numbers for 5 or 6 weeks, and at their maximum they numbered hundreds of millions per ml. Continued exposure to temperatures of 29 to 31°F causes the formation of small ice crystals which gradually increase until the milk is filled with them; however, the milk does not freeze solidly. In spite of the fact that the milk was a semi-solid mass of ice crystals, the enormous increase in bacteria took place. It was not until the bacterial content began to fall and the organisms of putrefaction were at work that the use of the milk for household purposes would, to the ordinary observer, become contraindicated.

In summary the author concludes that in general for approximately 6 to 8 days, bacteria in milk are quiescent if storage is proper (0°C); however even under storage at this temperature after this time there is a steady increase until very great numbers of bacteria are present.

15. Ravenel, M. P., Hastings, E. G., and Hammar, B. W.
1910. The Bacterial Flora of Milk Held at Low Temperatures. J. Infect. Dis., 7:38-46 (Bacteriological Laboratories of the University of Wisconsin.).

Experimental data shows that milks held at -9°C show no increase in bacteria developing on agar and gelatin; however, there was a clumping of casein and fat, an increase in the amount of soluble nitrogen and a decrease in the acidity. In milks held at 0°C there was a marked increase in the bacterial content resulting in an increase in acidity, an increase in the percentage of soluble nitrogen so that it eventually

amounted to over 70% of the total nitrogen, and a decrease in the total nitrogen content probably due to a liberation of free nitrogen.

The author reviews the earlier literature with regard to bacterial population in stored milk and discusses some of the needs and problems of storage of such products.

16. Mott, F. E. and Mozer, H.
1942. Deterioration of Milk by Bacterial Growth Under Refrigeration at 40°F. New England J. of Med. 227:174.

Grade A and Grade B pasteurized milk held for 72 hours at 40°F had such bacterial growth that their sale would violate the Massachusetts law. Certified pasteurized milk, even after 96 hours at 40°F, conformed to bacteriological standards.

17. Hartsell, S. E.
1944. Studies on the Bacteriology of Stored, Dried Egg Powder. Food Research, 9:505-511. (Dept. of Biology, Purdue University, West Lafayette, Indiana.).

The date indicates that as the storage temperature of spray-dried whole-egg powder is increased, the total bacterial count is decreased with time. Yeast-water (one part) added to the glucose-tryptone agar (nine parts) as recommended in Standard Methods for the Examination of Dairy Products, is a desirable medium for determing the total bacterial counts of stored, spray-dried, whole egg powder. The bacteria found most frequently in stored egg powder belong to the genus bacillus; their presence, however, may not be detected unless suitable plating media are employed. Data indicates that an incubation temperature of 32°C gives higher total counts and more successful isolations than at 37°C. Compression of spray-dried, whole-egg powder may reduce the total bacterial count slightly, but has no noticeable influence on the genera surviving in the stored samples. Storage in tin cans, greaseproof cartons, or greaseproof cartons plus carbon liners did not influence the bacterial flora of the stored samples.

The author cites evidence from his experience and from the literature on methods to be used to establish a satisfactory product.

18. Kiser, J. S. and Beckwith, T. D.
1942. Effect of Fast-Freezing Upon Bacterial Flora of Mackerel. Food Research., 7:255-259. (Biological Research Institute, San Diego, and the University of California, Los Angeles, California.).

Freezing and storage at -28°C for 10 days sharply reduces the bacterial flora of the muscle and intestinal contents of the mackerel. Freezing and storage for 48 hours at -28°C. produced a greater decrease in numbers in suspensions of bacteria isolated from mackerel than did similar freezing and storage at -20°C. Freezing and storage at -20°C. resulted in an approximate 100 % decrease in suspensions of Achromobacter sp. used; however, Micrococci withstood the low temperature much better.

19. Kiser, J. S. and Beckwith, T. D.
1944. Bacterial Flora of Mackerel (Effect of Fast Freezing). Food Research, 9:250-256. (Biological Research Institute, San Diego, and The Dept. of Bacteriology, University of California, Los Angeles, California.).

In this paper the authors point out that although numerous investigations of the flora of fish from marine waters have been previously made, this study will be important to the frozen food industry (especially on the industry of the West Coast.).

Qaulitative studies of 34 mackerel were undertaken. Heart, liver, back muscles, and the contents of the stomach and intestine were studied. Members of the following groups were the preponderant organisms: Micrococcus, Achromobacter, Pseudomonas, Flavobacterium, Sarcina, Kurthia, Lactobacillus, and Streptococcus. One strain of Escherichia was encountered.

Quantitative studies of the back muscles showed fewer than 1000 per gram in most instances, but the intestinal microorganic content was occasionally of the order of 10^7 per gram.

20. Kiser, J. S.
1944. Effects of Temperatures Approximating 0°C Upon Growth and Biochemical Activities of Bacteria Isolated From Mackerel. Food Research, 9:257-267. (Biological Research Institute, San Diego, and The Dept. of Bacteriology, University of California, Los Angeles, California.).

Organisms isolated from the mackerel were well adapted to growth at low temperatures. Eighty percent of the cultures produced macroscopic growth in 6 days at 0°C. At low

temperatures the physiological activities were not always characteristic of those at temperatures more nearly optimal. Growth curves, minimum generation times and temperature coefficiants are presented for an Achromobacter sp.

An important feature of this paper is the evidence presented to demonstrate the marked adaptability of these species.

21. Bisset, K. A.
1946. The Effect of Temperature on Non-Specific Infections of Fish. J. Path. Bact., Lond., 58:251-258. (Bacteriology Dept., University of Edinburgh.).

This is an interesting paper which indicates that fresh water fish may become parasitized by what are ordinarily saprophytic water bacteria. A rise in temperature of the water from 10 to 23°C in the case of goldfish will upset the balance between the host and these ordinarily harmless bacteria. When this upset occurs there is at first an increase in the number of bacteria in the tissues of the fish (which may result in death); however, if the fish survives the number declines and the tissues become cleared of infection. Because of this increased antibacterial defense at higher temperatures, the fish are able to withstand an initial infection more successfully; on the other hand, at low temperatures the efficiency of these defenses is so low that even organisms which appear devoid of pathogenicity towards fish may be harbored over a period of some weeks.

22. Aschehoug, V. and Vesterhus, R.
1947. Bacteriological Investigation on Spoilage of Winter Herring During Storage. Food Research, 12: 55-76.

This paper is an important one from the standpoint of the frozen fish industry. The normal bacterial flora of the fish is discussed along with the effect of storage on the spoilage of the fish.

23. Weinzirl, J. and Newton, E. B.
1915. The Fate of Bacteria in Frozen Meats Held in Cold Storage and Its Bearing on a Bacteriological Standard of Condemnation. Am. J. Pub. Health 5: 833-835 (University of Washington, Seattle.).

In This research the authors held 10 samples of hamburger steak until all of them showed a positive organoleptic test and also ammonia by Eber's test. A bacteriological analysis was then done following which the samples were frozen and stored at -10°C. The following data shows the

the remarkable decrease in organisms after freezing and storage. (Average counts from the 10 samples are presented).

July 16, 1913 - - - -	192,000,000
July 29, 1913 - - - -	37,000,000
Nov. 9, 1913 - - - -	23,000,000
Apr. 11, 1914 - - - -	13,000,000
July 29, 1914 - - - -	8,000,000

The authors conclude that a bacterial standard alone cannot be applied to frozen meats for all of the samples studied showed advanced putrefaction before they were placed in the refrigerator.

24. Hoffstadt, R. E.
1924. Bacteriological Examination of Ground Beef with Reference to Standard Analysis. I. Relation of Bacterial Count and Aerobic Species Present to Spoilage. Amer. J. Hyg., 4:33-42. (Bacteriological Laboratory, School of Hygiene and Public Health, Johns Hopkins University.).

The author believes that the possibility of using aerobic bacterial counts and aerobic bacteria as a standard of meat analysis can be eliminated. In his studies, the bacterial count of all samples shows much variation in relation to sanitary environment and the organoleptic test. No relation between the initial bacterial count and that of aging meat was noticed. Of 645 aerobic organisms isolated, 328 were of environmental origin, 317 of fecal or doubtful origin.

25. Hoffstadt, R. E.
1924. Bacteriological Examination of Ground Beef with Reference to Standard Analysis. II. Anaerobic Species Present in Ground Beef and Their Relation to Spoilage. Amer. J. Hyg., 4:43-51. (Bacteriological Laboratory, School of Hygiene and Public Health, Johns Hopkins University.).

In this study some 347 anaerobes were isolated and identified. The conslusion drawn is that the presence of proteolytic anaerobes indicates a definite way by which the keeping qualities of meat can be predicted. A technique for such determinations is suggested.

26. Prescott, S. C., Hale, F. J., and White, G. E.
1931. Observations on Bacteriology of Slimy Beef. J. Bact., 21:26.

The authors describe a rapid superficial development of microorganisms on beef which gives rise to disagreeable odors and a slimy coat. The important factors in production of slimy beef are (1) a temperature just above freezing, (2) a high humidity, and (3) an insufficient air exchange.

27. Noble, and Hardy, F.
1945. Effect of Storage Temperature and Time Upon the Quality of Pork Preserved by Freezing. Food Research, 10:165-175.

"Frozen pork loin roats, even obtained from high grade animals and carefully handled, cannot be stored at -18 to -9°C for longer than 16 to 22 weeks without danger of having the flavor of the fat and the aroma decrease to a point commonly described as slightly desirable."

28. Gross, C. E., Vinton, C., and Martin, S. Jr.
1946. Bacteriological Studies Relating to Thermal Processing of Canned Meats; Viability of Spores of a Putrefactive Anaerobis Bacterium in Canned Meat after Prolonged Incubation. Food Research, 11:399-404. (Research Laboratories, John Morrell and Company, Ottumwa, Iowa.).

The authors demonstrated viable spores in cultures of tubes showing no visable spoilage after incubation for one year at 28°C.

29. Gross, C. E., Vinton, C., and Stumbo, C. R.
1946. Bacteriological Studies Relating to Thermal Processing of Canned Meats; Characteristics of a Putrefactive Anaerobe Used in Thermal Resistance Studies. Food Research, 11:405-410. (Research Laboratories, John Morrell and Company, Ottumwa, Iowa.

Putrefactive anaerobes S_2 and 9_x isolated as the causative agents of spoilage in canned meats are culturally and serologically identified with P. A. 3679 (a test organism frequently used by the NCA laboratories for inoculated pack studies.

30. Vinton, C., Martin, S. Jr., and Gross, C. E. 1947. Bacteriological Studies Relating to Thermal Processing of Canned Meats; Effect of Substrate Upon Thermal Resistance of Spores. Food Research, 12:173-183. (Research Laboratories, John Morrell and Company, Ottumwa, Iowa.).

It has been demonstrated for P. A. 3679 in meat, organs, mixtures of meats, and for mixtures of meats and organs that the thermal resistance of spores is less when grown in raw meat or organs than when grown in either pasteurized or sterilized meats and organs. The effect is shown to be related to the substrate itself through repeated raising and lowering of resistance by transfer from media to media. (Note: it would be interesting and of value to know whether or not there is a similar effect when cold is considered.).

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